Toxicant Profile for Polychlorinated Biphenyls: New Bedford Harbor

Prepared by:

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1.0 EXECUTIVE SUMMARY

1.1 INTRODUCTION

The commercial production of polychlorinated biphenyls (PCBs) began in 1929 in response to the electrical industry's need for flame resistant dielectric insulating fluids for use in capacitors and transformers. PCBs were useful in a number of industrial and domestic applications because of their unique functional characteristics including thermal stability; resistance to acids, bases, and chemical reactions; low water solubility; high electrical resistivity; suitable viscosity-temperature relationships and flame retardant properties. Although the primary need for PCBs was as insulating fluids in electrical capacitors and transformers, their use widened to include applications in heat transfer fluids; hydraulic, vacuum pump and compressor fluids; lubricants; as a plasticizer in adhesives, textiles, surface coatings, and sealants; caulking compounds; carbonless paper systems and waxes.

During the 1960s there was increasing awareness of organochlorine pesticide contamination in the environment, beginning particularly with DDT. The search for pesticide contamination accidentally led to the discovery by the late 1960s that PCBs had become widespread environmental contaminants in the United States and elsewhere. In 1968, public concern over the possible health effects of PCBs increased following the "*Yusho*" incident in Japan. As a result of a Japanese industrial accident, about 1,000 people became ill from eating rice oil heavily contaminated with Japanese-produced PCBs (Kanechlors). Though it was later found that the Yusho symptoms were probably caused by polychlorinated dibenzofuran contaminants, the incident sounded a cautionary signal which prompted legislative actions in many countries to limit the production and use of PCBs.

Following reports of the Yusho PCB incident in Japan, the United States Food and Drug Administration (FDA) initiated a nationwide survey to investigate the extent of contamination of PCBs in food. The FDA found evidence of widespread food contamination which had probably occurred primarily through the use of PCB-contaminated animal feed and PCB-containing paper

food-packaging materials. In response to these findings, the FDA established guidelines limiting the levels of PCBs in foods and food-packaging products which contain unavoidable residues from environmental and industrial sources. In 1970, cautionary measures were also taken by the U.S. manufacturer of PCBs. These measures included limiting the sale of PCBs to *"sealed system"* uses only, as well as steps to ensure their safe shipment and prevent further environmental contamination. Since 40% of the PCB sales prior to 1970 had been for *"open system"* uses, e.g. plasticizers, hydraulic fluids and lubricants, this action substantially decreased PCB production and usage in the United States. Still, the federal Interdepartmental Task Force on PCBs concluded in 1972, *"Their continued use for transformers and capacitors in the near future is considered necessary because of the significantly increased risk of fire and explosion and the disruption of electrical service which would result from a ban on PCB use."*

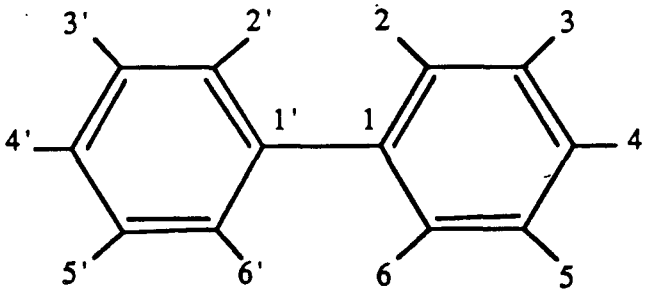
By 1976, the electrical industry developed suitable alternatives for PCBs; silicone compounds were adapted for use in transformers and phthalate esters were used in capacitors as PCB substitutes. In 1976 Congress passed the Toxic Substances Control Act which specifically banned the manufacture of new PCBs and prohibited use of existing PCBs except in a *"totally enclosed"* manner or where specifically exempted. United States manufacturers terminated the production and sales of PCB products by September, 1977, and the PCB *"banning"* legislation became effective in 1979.

In spite of these measures, the problem of environmental PCB contamination is far from solved. The tremendous popularity of PCBs in previous years resulted in the production of some 1.4 billion pounds of various PCBs by 1977. An estimated 750 million pounds of PCBs are still in use today, 20% (or 162 million pounds) of which are associated with electrical utilities and are found in many existing capacitors and transformers. Eliminating even the majority of the PCBs still in use today would be an imposing task. Of the PCBs no longer in use, an estimated 300 million pounds were placed in landfills, 150 million pounds were exported, 50 million pounds were destroyed, and another 150 million pounds were dispersed in other areas of the environment.

The PCB production in the United States represented only a fraction of the total world PCB production. NIOSH (1977) reported PCB production in France, Italy, the Soviet Union, Spain, Czechoslovakia, Poland, Argentina, Brazil, and India. In fact, PCBs are still produced in some of these countries. Therefore, the production, use, and environmental occurrence of PCBs, will undoubtedly continue for many years.

1.2 THE CHEMICAL AND PHYSICAL PROPERTIES OF PCBs

PCBs are a group of synthetic organic compounds with the empirical formula $C_{12}H_{10-n}Cl_n$ (where n = 1-10). Each PCB molecule consists of two benzene rings bound to each other at what has been designated as the 1 position of each ring to create a biphenyl structure. The basic chemical structure of a PCB molecule is depicted in Figure 1.1.1. Carbon atoms in the rings not involved



Polychlorinated Biphenyl

Figure 1.2.1

in the inter-ring bond have either a hydrogen or chlorine atom attached. The ten sites on the basic biphenyl moiety that might be chlorinated create the possibility of 209 distinct, individual PCB congeners, though three of these would technically be considered mono- rather than poly-chlorinated biphenyls. While 209 different PCB

congeners exist, not all are likely to occur at significant levels in commercial PCB mixtures. Indeed, it has been reported that some 20 individual PCB congeners have not been found during analysis of the commonly produced commercial PCB mixtures.

Individual PCB congeners are numbered as shown in Figure 1.1.1. The two carbon atoms forming the single bond between the phenyl groups are numbered 1 and 1'. The two rings are numbered identically, except that the ring which contains the carbon labeled 1' is numbered in primed numbers according to the IUPAC definitive rules. These rules are as follows:

- an unprimed number is assigned a lower order than the corresponding primed number (i.e. 2 is lower than 2');
- a lower number is assigned to a point of attachment in equivalent positions (i.e. 2 versus 6 for a substituent in one of the *ortho* positions); and
- the ring with the greatest number of substituents is unprimed, and when the number of substituents in the two rings is the same, the unprimed numbers are assigned to the ring containing chlorines in the lowest-numbered positions.

The nomenclature of various commercial PCB mixtures differs according to the manufacturer. In the United States, the most widely used commercial PCB mixtures were those manufactured by Monsanto under the tradename Aroclor®. Different Aroclor mixtures were designated by a four-digit number such as 1242, 1254, 1248, and 1260, etc. The first two digits in this nomenclature denote the fact that the biphenyl nucleus contains 12 carbons, while the latter two digits indicate the average weight percentage of chlorine in the mixture. An exception to this rule was Aroclor 1016, which had 41% chlorine, not 16% as its name implies. The content of penta- through hepta- chlorobiphenyls had been significantly reduced in Aroclor 1016, and its atypical designation emphasized that its composition differed significantly from Aroclor 1242 despite its similar chlorine content.

While most of the individual PCB congeners are solids at room temperature, commercial mixtures (particularly Aroclors 1016-1254) can be mobile oils or viscous liquids. The fluidity of PCB mixtures arises from the fact that the combination of individual congeners acts to depress their melting

points. For this reason, most PCB mixtures do not crystallize, but show a "*pour point*" below which the mixture changes into a resinous state.

The physical properties of PCB congeners and commercial mixtures vary greatly depending on the degree and position of chlorine substitution. In general, with increasing chlorine content (e.g. increasing Aroclor number), there is an increase in the density, flash point, fire point, pour point, and distillation range of the PCB mixture. On the other hand, the dielectric constant and water solubility vary inversely with chlorine substitution. This decrease in the dielectric constant corresponds to an increase in electrical insulating properties.

Other important properties of PCB isomers and commercial mixtures such as solubility, vaporization and vapor pressure, and lipophilicity also vary with the degree and position of chlorine substitution. In general, the water solubility and vapor pressure of the PCB mixture decrease as the degree of chlorination increases. In contrast, the lipophilicity of the PCB congener tends to increase with the degree of chlorination. Individual chlorobiphenyls vary in their solubility from about 6 parts per million (ppm) for 2-monochlorobiphenyl to as low as 0.007 ppm for octachlorobiphenyl. The position of the chlorine substitutions may also have some impact on the water solubility of the PCB congener. For example, the solubility of 2,4'-dichlorobiphenyl is 1.88 ppm while that of 4,4'-dichlorobiphenyl is only 0.08 ppm. As the lesser-chlorinated congeners are generally more soluble than the more heavily-chlorinated congeners, chromatographic analysis of aqueous solutions of Aroclors shows greater proportions of the lower chlorinated fraction than exist in the standard Aroclors from which the solutions are made.

The above-mentioned aqueous solubilities represent those determined experimentally, while the solubilities of Aroclor mixtures in environmental samples of water can vary greatly depending upon certain conditions. This variability may be explained by the fact that environmental samples of water generally contain dissolved organic substances which may increase the apparent water solubility of the PCBs in the sample.

The vaporization rate of PCBs is minimal at most ambient temperatures, being on the order of only 10^{-3} to 10^{-5} mm of Hg at 25° C. In fact the vaporization rates (in $gm/cm^2/hr$) of PCB mixtures heated to 100° C range from only 0.00174 for Aroclor 1221 to 0.00009 for Aroclor 1260. The vapor pressure and vaporization rate of PCBs in environmental soil and sediment samples are typically lower than those measured under laboratory conditions. This is thought to result from the adsorption of PCBs onto soil or sediment surfaces. Although not completely understood, differences in vaporization rates from various soils must be due to differences in soil or sediment factors such as surface area, organic matter content, and pH.

Unlike the vaporization rate of PCBs from soil or sediment, the vaporization from aqueous solutions is much higher than one would expect based on their low vapor pressure and high molecular weight. Thus, the evaporation of PCBs from contaminated rivers and lakes represents a major means for their environmental transport. The transport and fate of PCBs in aquatic systems may depend on a number of factors influencing adsorption reactions. Generally speaking, the adsorption of PCB congeners and mixtures to soils and sediments increases with increasing chlorine content, surface area, and organic carbon content of the matrix. Empirical evidence clearly demonstrates that PCBs are strongly held to the surfaces of soil adsorbents and that PCBs adsorb to soils remain relatively resistant to leaching by water.

1.3 TOXICOLOGY OF PCBs IN ANIMALS

1.3.1 Pharmacokinetics

Although detailed bioavailability studies are generally lacking, it appears that significant absorption of PCBs may occur for all routes of exposure in animals. Studies measuring the extent of absorption of ingested PCBs have generally found that 90% or more of the dose was absorbed. Absorption of PCBs may also occur through the skin, and studies have found that the dermal absorption of some PCB commercial mixtures over a 24-hour interval in the guinea pig and monkey ranges from 15-56%. Very little is known about the

absorption of PCBs from inhalation. PCBs have been measured in the livers of rats after inhaling a PCB-containing aerosol, indicating that some absorption through the lungs had occurred. There are no data to indicate the extent of absorption by inhalation in animals, however.

Following ingestion PCBs probably enter the vascular space via the lymphatic system bound to chylomicrons. Once they have entered the bloodstream, their extremely low aqueous solubility dictates transport primarily bound to lipoproteins, albumin, cellular elements and chylomicrons. Following absorption PCBs are fairly rapidly transported to all tissues, where tissue distribution is driven by the perfusion rate of the organ and the tissue's affinity for PCBs. As a consequence, PCBs are initially taken up by the liver (high perfusion rate and affinity) and muscle (high perfusion rate and largest percentage of body mass). This phase is followed by a re-distribution driven primarily by the lipid solubility of PCBs. During re-distribution PCBs move toward organs of highest affinity (but low perfusion rates and hence low initial uptake) like the skin and adipose tissues. It is apparent from the literature that both the extent and position of the chlorination profoundly influence the distribution and elimination of individual PCB congeners, and that there can be important species differences in the disposition of PCB congeners.

The ability to excrete PCB molecules is limited by their water solubility, which is very low. Therefore, in order to be effectively excreted, PCBs must be converted to more water soluble forms through metabolism. Hydroxylation is the predominant metabolic reaction and occurs primarily through the formation of arene oxide intermediates. The arene oxide-forming step essentially requires two adjacent, unsubstituted (i.e., non-chlorinated) carbon atoms, and is thought to be responsible for hydroxylation at the 4 and 4' (*para*) or 2 and 2' (*ortho*) positions on the PCB molecule. Hydroxylation may also occur through a direct insertion mechanism, although this is probably important only for hydroxylation at the 3 or 3' (*meta*) positions. The linear nature of the molecule tends to direct metabolism toward the ends of the molecule, and hydroxylation at the 4 or 4' (*para*) positions occurs most readily. Increasing chlorination limits the number of carbon atoms available for hydroxylation, and higher-chlorinated PCB congeners are generally metabolized more slowly. The

requirement of two adjacent, unsubstituted carbons for arene oxide formation explains why the position of chlorination is also important in influencing the rate of PCB metabolism. For example, congeners without unsubstituted carbons at the ends of the molecule, such as 3,3',4,4'-tetrachlorobiphenyl, are slowly metabolized and therefore slowly eliminated.

Because individual PCB congeners may have different inherent rates of metabolism and elimination, the ratios of congeners present in the body after a dose of a PCB mixture will change with time. In general, those congeners more difficult to metabolize and excrete (usually higher-chlorinated PCBs) will with time comprise a greater fraction of the PCBs remaining from a dose. It should also be noted that metabolism does not always enhance elimination of PCBs. The arene oxide intermediates of some congeners may become conjugated with glutathione. Gut microbes degrade these conjugates to methylthio- and methylsulfonyl- metabolites which may be reabsorbed and persist in certain tissues like the lungs.

The rate of PCB metabolism may vary significantly with species. In general, the rate of PCB metabolism among species declines in the following order : mammals > birds > fish. Among mammalian species the order is dogs > rats and mice > monkeys and mink.

1.3.2 Acute and Subchronic Effects

The acute toxicity of PCBs is sufficiently low as to be described as "*slightly toxic*" or "*practically non-toxic*" by American Industrial Hygiene Association standards. This is based on the fact that in order to produce acute lethality one must administer doses above 1,000 mg/kg to most species. PCB toxicity is time-dependent, however, and the magnitude of the single dose required to produce lethality decreases as the observation period increases. In rats, the toxicological and pathological findings at acutely lethal doses include anorexia, central nervous system depression, hepatotoxicity, nephrotoxicity, coma and death.

A number of toxic effects are observed with subchronic exposure,

principally involving the liver, skin, and immune system. PCB effects on the liver include hypertrophy, enzyme induction, lipid vacuolization (fatty liver), and necrosis. Enzyme induction, the increase in content or activity of metabolic enzymes, and hypertrophy are related effects and may be viewed as an adaptive response which accelerates the rate of metabolism and elimination of PCBs and other compounds. Enzyme induction is not a toxic effect, *per se*. Because the metabolism of compounds other than PCBs may be affected, PCBs may modify the extent or duration of effects of some other substances. Although it is difficult to generalize the outcome of enzyme induction-based interactions between PCBs and other compounds, PCB enzyme induction appears to decrease the tumor response to a number of carcinogens. Fatty liver has been commonly associated with PCB administration in a number of species, and some studies have found single cell or focal necrosis. Neoplastic changes in the liver in animals following chronic PCB administration are discussed separately under Genotoxicity and Carcinogenicity, below.

In addition to effects on the liver, a variety of immune abnormalities has been observed in animals following PCB administration which in general indicate a decreased functional capability. This has been indicated by both measurement of specific parameters related to immune system function and by studies indicating impaired response to challenge with infectious agents. The immune system does not seem to be especially sensitive to PCBs, however, and diminished immune system capability has only been demonstrated at relatively high PCB doses.

The skin is also a common site of PCB toxicity. While dermal effects of PCBs may manifest themselves somewhat differently among species, they are generally the result of hyperplasia and hyperkeratosis. In the monkey, acne-like lesions are produced that resemble the chloracne reported after high exposure to PCBs in humans.

A number of biochemical effects in animals or animal tissues have also been reported to be caused by PCBs. PCB administration has been associated with decreases in hepatic retinol and serum thyroxine levels, altered lipid metabolism leading to elevations in serum levels of some forms of cholesterol

and triglycerides, and alterations in porphyrin metabolism leading to porphyria. The extent to which any of these changes contributes to morbidity with PCB administration has not been established, however. Potential PCB effects related to reproductive function or teratogenicity, or related to genotoxicity and carcinogenicity, are discussed separately below.

1.3.3 Mechanism of PCB Toxicity

Despite considerable study, little has been established regarding the mechanism by which PCBs produce their toxic effects. The metabolism of some PCB congeners has been shown to result in the formation of reactive, covalently-binding metabolites and this has been proposed as a possible mechanism for at least some toxic effects of PCBs. However, this does not appear to be a likely mechanism of PCB toxicity in that structural features of PCB congeners (i.e. degree and position of chlorination) that favor this metabolite formation are those associated with less toxicity.

Much more attention has been given to a proposed mechanism of toxicity in which the binding of PCBs to a cytosolic receptor initiates the toxicity. Among inbred mouse strains there is generally a good correlation between binding to this receptor, called the *Ah* receptor, and a number of toxic effects of PCBs. While binding to this receptor may play a role in some toxic effects of PCBs, three lines of evidence indicate that *Ah* receptor binding is unlikely to be the critical event in the development of PCB toxicity. These are: 1) the fact that a number of studies have shown a dissociation between enzyme induction in response to *Ah* binding and PCB toxicity; 2) the fact that there is a lack of correlation between receptor binding and the toxicity of halogenated aromatics like PCBs and TCDD among species; and 3) the fact that there is a lack of correlation between receptor binding of halogenated aromatics thought to produce toxicity via the *Ah* receptor and their toxic potency within a particular species. Thus, when all the data are considered, the correlation between *Ah* receptor binding or stimulation and organ toxicity is poor. It has been proposed that the *Ah* receptor merely functions as a transport protein, carrying the PCB molecule to the nucleus where it interacts with another receptor to initiate the toxic event. The nuclear thyroxine-binding site has been suggested to be this

receptor, although it is unclear precisely how even prolonged stimulation of this receptor would result in the characteristic PCB effects.

1.3.4 Reproductive Toxicity and Teratogenicity

The range of reported effects on reproduction in animals includes a lengthening of the estrus cycle, weak estrogenic activity and persistent vaginal estrus, fetotoxicity and fetal deaths, decreased survival of the neonate, small-for-gestational-date birth weights, and teratogenic effects. There is considerable species variation in the responses generally reported. Among the species of laboratory animals tested, rats and mice appear to be more resistant species while mink and monkeys appear to be relatively sensitive. While mink and monkey studies tended to be of longer duration, these differences in sensitivity cannot be attributed solely to the length of the study. A two-generation study in rats found dietary levels as high as 5-20 ppm to be without significant reproductive toxicity, and yet these dietary levels in mink and monkeys resulted in complete reproductive failure and significant maternal mortality. Some of the differences in sensitivity might be attributed to differences in metabolism, as mink and monkeys do not appear to eliminate PCBs as rapidly as other species. However, the body burdens achieved in less sensitive species during some of these studies have been substantial, so variations in the inherent reproductive toxicity of PCBs among species appear to exist.

Mink and monkeys have suffered reproductive problems from chronically-fed, dietary PCB levels as low as 2-2.5 ppm. In both mink and monkeys obvious signs of maternal toxicity preceded or were present when reproductive problems occurred, and in studies with each species the doses generally tested were such that a significant portion of the maternal test population died before giving birth. The fact that reproductive problems seem to occur at or near maternally-toxic doses in these sensitive species is illustrated by the fact that the dietary LC_{50} for Aroclor 1242 in mink is 8.6 ppm and yet dietary levels of 2 ppm in the diet have been reported to be without effect on mink reproductive success. In the rat, a less sensitive species, adverse reproductive effects are generally associated with exposure to dietary PCBs at levels of 100 ppm or greater. This is greater than the

PCB levels associated with other organ toxicities such hepatotoxicity, indicating that the reproductive system is comparatively less sensitive to toxic effects of PCBs.

Studies in rats, mice, mink and monkeys indicate that only a small percentage of the maternal PCBs cross the placenta and are absorbed by the fetus. Studies to date suggest that little in the way of adverse reproductive effects would be expected if no significant maternal toxicity is produced by the dose. However, during lactation a large portion of the maternal PCB body burden may be eliminated via breastmilk, and the neonate may receive an acutely lethal dose of PCBs in this manner.

A number of studies designed primarily to investigate the reproductive toxicity of PCBs have been evaluated without any evidence of terata being reported. This suggests that at least no gross, physical defects were caused by PCB administration in these studies. More recently, however, a few studies have examined the teratogenic effects of PCB mixtures or single PCB congeners in mice and other species. In these investigations, morphological or neurobehavioral effects were studied as teratogenic endpoints. Although some studies are regarded by their authors as positive evidence of the teratogenicity of PCBs, maternal toxicity or limitations in the experimental design of these investigations severely limit the relevance of these findings. For example, the doses used in each case were relatively high, and in fact the doses of some of the isomers tested cannot be achieved through sub-lethal exposure to a commercial PCB mixture. Therefore, while there is evidence which suggests that certain hexachlorobiphenyl and tetrachlorobiphenyl PCB congeners are teratogenic in mice, there is no reasonable evidence that commercial PCB mixtures are teratogenic at doses not producing significant maternal toxicity or mortality.

Some studies have examined interactions between PCBs and tetrachlorodibenzodioxin (TCDD) using reproductive endpoints. In one study, the incidence of TCDD-induced cleft palate in mice was enhanced by 2,3,4,5,3',4'-hexachlorobiphenyl but not by 2,4,5,2',4',5'-hexachlorobiphenyl. In contrast, co-administration of a commercial PCB mixture, Aroclor 1254, with TCDD significantly reduced the incidence of cleft palate induced by TCDD alone.

It appears that some PCB congeners are capable of enhancing the teratogenicity of TCDD while others, including congeners present in Aroclor 1254, can substantially diminish its teratogenicity.

1.3.5 Genotoxicity and Carcinogenicity

The results of the PCB genotoxicity studies have been summarized in Table 3.6.1. Taking into consideration the number of times PCBs have been tested in a reasonable battery of genotoxicity or mutagenicity tests and found to be without activity, and the fact that the tests selected measure several different critical endpoints of genotoxicity, it appears that there is no scientific evidence of significant genotoxic activity for commercial-grade PCB mixtures. It is also concluded that these studies demonstrate that PCB-induced liver tumors observed in rodents most probably occur by an epigenetic mechanism.

The investigation of PCB carcinogenic potential in mice is limited to two short studies, while approximately eight to ten studies have been reported using various strains of rat. The PCB mixtures tested thus far are: 1) in mice- Kanechlor 300, Kanechlor 400, Kanechlor 500 and Aroclor 1254; and 2) in rats - Kanechlor 300, Kanechlor 400, Kanechlor 500, Clophen A30, Clophen A60, Aroclor 1254 and Aroclor 1260.

Tests of Kanechlor mixtures in mice were negative except for mice treated with the highest dose of Kanechlor 500 (500 ppm). Hepatic tumors in these mice were inconsistently reported as either hepatomas or carcinomas in different publications. The absence of tumorigenesis from lower doses of Kanechlor 500, and all doses of the lesser-chlorinated Kanechlor 300 and 400 in mice suggests that the tumorigenic response was both dose- and chlorination-dependent. A study of Aroclor 1254 in mice found hepatomas associated with dietary exposure of 300 ppm for 11 months, but almost no effect if the PCBs were given for six months with a five-month recovery period.

In rats, Aroclor 1260 or its equivalent, Clophen A60, has produced hepatocellular carcinomas in three studies at doses of approximately 100 ppm, a

dose which appears to represent the maximally-tolerated dose for rats. A review of these three studies indicates that the tumors occurred very late in the life of the animal, with a significant incidence of tumors only beginning to appear after about two years of exposure. Of interest is the fact that PCB treatment, while increasing the incidence of liver cancer, did not increase the total cancer incidence. Increases in hepatic cancer incidence were offset by decreases in other types of cancer. The suggestion that PCBs may have some antitumorigenic activity has been supported by studies measuring PCB effects on transplanted Walker 256 sarcoma in rats. The effects of chronic 60% chlorine PCB treatment were not life-shortening, and in fact in two of the studies the morbidity and mortality of the animals were decreased by PCB treatment. Though the hepatic tumors were classified as carcinomas, they did not display the aggressive, invasive behavior associated with malignant neoplasms. PCB mixtures of lesser chlorination, Aroclor 1254 and Clophen A30 (similar in PCB composition to Aroclor 1242), have been examined in chronic bioassays in rats with no significant increase in cancer observed. Thus, conclusions to be drawn from the rat data, like the mouse data, appear to be specific for degree of chlorination of the PCB mixture.

Perhaps because PCBs produce liver hypertrophy, they enhance the tumorigenesis of certain liver carcinogens if given after the carcinogen in question has had the opportunity to initiate tumors. However, dose-response studies for this promoting effect indicate that it occurs at doses in excess of reasonable human exposure. If the PCB exposure precedes or is concomitant with exposure to liver carcinogens, the tumorigenic response is typically decreased, probably as a result of enhanced metabolic detoxification of the carcinogen.

1.4 HUMAN HEALTH EFFECTS OF PCBs

1.4.1 Routes of Human Exposure and Human Tissue Levels

Given the widespread commercial application of PCBs, it is not surprising that human exposure to PCBs has occurred in a variety of contexts. While workers involved in the industrial uses of PCBs may have been exposed to relatively high levels for prolonged periods of time, exposure of the general population to lower levels of PCBs has also been a constant feature of our society. For example, rural air contains PCBs at concentrations in the neighborhood of 2-20 $\mu g/m^3$, urban air may range from 20-100 μg PCB/m^3, and indoor air may be reasonably expected to contain some 100-500 μg PCB/m^3 regardless of whether it is in a home, school or office. By comparison, drinking water and food probably currently represent lesser routes of incidental PCB exposure. The limited data available suggest that only a small percentage (probably < 3%) of finished drinking water supplies contain quantifiable levels of PCBs (0.1-2.0 ppb). Because low levels of PCBs can be found in all fish caught within U.S. waters, fish consumption, like air, remains a low-level but unavoidable source of exposure today. Other foods also may contain small amounts of PCBs, though the levels appear to be declining since the mid to late 1970s.

As it became obvious that most persons living in the U.S. and other countries were exposed daily to small amounts of PCBs, it also became obvious that persons living in these countries should have some level of PCB residue within their tissues, particularly fatty tissues. Within the last decade a number of studies have verified that essentially all persons within the U.S. contain measurable levels of PCBs. Typically the average PCB serum level for persons having no unusual environmental or occupational exposure to PCBs is perhaps as low as 5-10 ppb. However, the normal range for individuals exposed only to ambient levels would seem to be at least as high as 40 ppb (see Section 4.2), and even higher values have been reported in a few studies. For fat tissue the upper end of the normal range is less clear but the number of *"nonexposed"* persons with levels of ≥ 3 ppm has ranged from about 1-10% during the last decade. Studies in the mid 1970s suggested the mean adipose PCB concentrations in

persons from Germany, Australia, and Japan may have been as high as 6.8, 3.5 and 7.5 ppm, respectively. Very recent evidence, however, indicates the average level in Japanese is now only about 1 ppm, and no doubt the current PCB level in residents of the United States is near this value.

Because of its high fat content, measurable levels of PCBs have also been found in human breast milk. As early as 1971, PCBs were identified in 20% of breast milk samples taken from women living in Colorado, with PCB levels ranging from 40-1000 ppb. By the late 1970s a national study indicated 69% of breast milk samples contained detectable levels of PCB, and 30% had values ranging from 50-4091 ppb (on a whole milk basis) with a mean value of 87 ppb. Based on 1,057 samples ranging from trace levels of contamination to 5.1 ppm (on a fat weight basis), the mean PCB breast milk concentration was 1.5 ppm, a value which corresponds to the FDA maximum allowable level in cow's milk. The presence of PCBs and organochlorine pesticides has been detected in human breast milk from other countries as well (see Table 4.2.2). In a number of countries the percentage of samples with detectable levels of PCBs was nearly 100 percent, and the reported levels range from <1-510 ppb on a whole milk basis. Given this information, it is clear that everyone's daily exposure to PCBs begins no later than birth, and estimates suggest a nursing infant will have a PCB fat level of 0.89 ppm by eight months of life.

Following unusually high exposure to PCBs, the level of PCBs in human tissue will return to background levels with time. While there have been no rigorous studies of PCB pharmacokinetics in man, the data from a number of studies can be used to provide an approximation of the human rate of elimination of PCBs. The rate at which tissue levels decline appears to be congener-dependent and influenced by the extent and position of chlorination. While the reported half-life for PCBs varies among the studies, an overall half-life (for total PCBs) of approximately six months was the most consistently reported value.

1.4.2 Clinical Findings Following Environmental Exposure

In the late 1960s and 1970s there were two incidents of large scale poisoning with rice oil contaminated with PCBs. One of these incidents occurred in Japan and the other in Taiwan. It is the conclusion not only of this review, but also of the scientific community, that the symptoms associated with these rice oil poisonings were the consequences of PCDF intoxication. They are not, therefore, instructive as to the potential adverse human health effects of PCBs (See Appendix A for further discussion).

A number of studies have attempted to determine the effects of PCBs from environmental exposure on reproduction and the health of the offspring. Using a variety of approaches, associations between maternal PCB levels and premature deliveries, spontaneous abortions, missed abortions, birth size and weight of neonate, behavioral deficits in the neonate, and susceptibility to illness in early life of the offspring have been sought. While some positive associations have been noted, other studies of similar design have failed to find any associations. In addition to this important inconsistency, serious flaws in study design have generally precluded the derivation of any cause-and-effect relationships. In many studies, *"exposed"* groups were poorly matched with controls with respect to important variables such as maternal age, smoking, alcohol consumption, etc. Additionally, some of these recent studies, such as those of women who ate contaminated Lake Michigan gamefish, were unable to demonstrate that their exposed populations in fact had significantly higher PCB body burdens than the control populations to which the comparisons were being made. If PCB body burdens do not vary significantly, it would seem that the associations derived from these studies are not likely to be caused by the measurable levels of PCBs present in us all. Most studies also failed to measure in their subjects the other organochlorines compounds typically found in the fish consumed. It is well known that elevated PCB levels in fish and humans usually correlate with elevated levels of other persistent organochlorines compounds such as DDE. It is therefore impossible to know if an effect associated with a particular PCB level is in fact due to the PCBs or to another, similarly ubiquitous environmental agent. The importance of this consideration is reinforced by the fact that those few studies that considered DDE levels and

corrected for this confounder failed to identify any effects that could be attributed to PCBs. In view of these many study limitations, it cannot be concluded at this time that environmental exposure to PCBs results in any adverse effect on human reproduction or fetal and neonatal health.

Relatively few clinical studies have examined persons with truly significant environmental exposure. Typically, the exposed populations had serum PCB levels ranging from normal to slightly elevated. Given the apparent limited increase in PCB dose, particularly when compared to magnitude of exposure among some occupationally-exposed individuals, it is perhaps not surprising that these studies provide limited information. The study populations examined have included persons eating PCB-contaminated fish in Michigan and Alabama, and residents exposed to PCB-containing sewage sludge marketed as fertilizer in Bloomington, Indiana. These studies have failed to identify any health problems associated with the PCB levels in these persons.

An additional finding of these studies that is of considerable interest is the indication that individuals living in areas of unusual contamination do not necessarily have significant PCB exposure. For example, the Greater New Bedford PCB Health Effects Study (MDPH, 1987) found no correlation between fish consumption and an elevation in blood levels of PCBs. Defining an elevated serum PCB level as ≥30 ppb, only 1.3% of the 840 participating residents of Greater New Bedford had elevated serum PCB levels. The subsequent "*Enrichment Study*" involving individuals with presumed heavier consumption of local seafood likewise revealed serum PCB levels within the typical range of the U. S. population. Therefore, the Greater New Bedford PCB Health Effects Study suggests that an increased potential for environmental PCB exposure does not necessarily result in elevated PCB body burden. Three studies involving potential exposure via contaminated soils provide similar results. One such study examined residents and workers in Bloomington, Indiana, after it was discovered PCBs had been discharged into the municipal sewage system by a local capacitor manufacturing plant. From the treatment plant sewage sludge was sold as fertilizer. Mean PCB concentrations were 479 ppm in sewage sludge, 17 ppm in fertilized soil, and 0.15 ppm in the vegetables grown in this

soil. However, the mean serum PCB levels in study participants were only 17.4 ppb in 89 sludge users in contrast to the 24.4 ppb measured in 22 community residents with no known exposure to PCBs. In another report, serum PCB levels in people living near three different contaminated waste sites who were thought to be at high risk of PCB exposure were only about 9 ppb (well within the normal range for *"nonexposed"* U.S. citizens). Last, the Agency for Toxic Substances and Disease Registry (ATSDR) conducted a detailed epidemiological investigation of the residents of Paoli, Pennsylvania, where widespread soil PCB contamination resulted through the long-term distribution of these substances from a highly contaminated rail yard. A *"preselected group"* residents (i.e., residential soil PCB measurements exceeding 150 ppm) had serum PCB levels that were not significantly different from the control population. ATSDR concluded, *"The population in Paoli near the work site experienced exposure similar to populations found elsewhere in the United States showing typical background exposures. ... There is no evidence of increased exposure in the groups closest to the most highly contaminated soil."* Thus, these studies indicate localized soil PCB contamination may not be an important source of PCB exposure and soil PCB levels are not predictive of the PCB dose actually received by residents in more heavily contaminated areas. They further reinforce the need to carefully evaluate whether the environmental exposure pathway being postulated is in fact complete.

1.4.3 Clinical Findings Following Occupational Exposure

The highest and longest PCB exposures to humans have occurred in the occupational setting. Serum PCB concentrations in workers may be roughly 100-times those observed in the general population, and the study of these workers clearly represents the best opportunity for determining adverse health effects of PCBs in humans. Several cohorts of occupationally-exposed workers have been examined for the presence or history of physical illness and have been subjected to a variety of clinical laboratory measurements. However, chronic exposure to PCBs in the occupational setting does not appear to produce any significant adverse human health effect other than dermatologic lesions, and the paucity of abnormal results in examined populations has been noted by several investigators. The two major effects often discussed in relation to

occupational exposure to PCBs are effects on liver function and serum triglyceride levels. The results of studies measuring the status of liver function in PCB-exposed workers were generally negative. When changes in parameters related to liver function were noted they were uniformly small and of questionable clinical relevance. Small changes in one clinical test of liver function were generally not supported by any of the other measures of liver function used in the same study. Such minor and inconsistent abnormalities would be anticipated in any large study of a healthy population, and are inconsistent with the hypothesis that PCBs have resulted in chemical-induced hepatotoxicity. Therefore, the data do not support the suggestion that the relatively high occupational PCB exposures occurring among capacitor workers resulted in liver injury. An elevation in serum triglycerides has been purported to be associated with serum PCB levels in some studies, and it was initially suggested that PCBs may alter lipid metabolism. However, studies of the distribution of PCBs indicate higher serum lipid levels increase the solubility of PCBs in serum samples. As a consequence, an elevation in serum triglycerides causes a greater percentage of an individual's PCB body burden to appear in the blood. The association between serum PCB levels and triglycerides therefore results from triglycerides influencing serum PCB levels rather than the reverse.

1.4.4 Mortality Studies

Several studies have examined the causes of mortality among workers occupationally exposed to PCBs. Given animal evidence of carcinogenicity for PCB mixtures of 60% chlorine, the cause of mortality of greatest interest has been cancer. In order for there to be human evidence of carcinogenicity for PCBs, it should be minimally expected that those persons with the largest known PCB exposure show an increase in death from cancer as compared to the reference cohort. Such an increase, if present, should be of sufficient magnitude as to not be explainable on the basis of chance alone, should show a positive relationship with the amount and duration of exposure, should show a positive relationship with the latency period, and should not be explainable on the basis of exposure to other carcinogenic substances. The evidence for carcinogenicity would be strengthened if specific types or sites of neoplasms

were consistently implicated, and if all confounding life-style related risk factors have been considered and eliminated. In reviewing the data from the mortality studies to date, none of these criteria has been met.

The largest study reported to date is one by Brown of the National Institute for Occupational Safety and Health. Both the overall mortality and the rate of mortality from cancer were less than expected. When neoplasms of questionable origin are removed from the analysis, there is no significant increase in cancer of the liver/biliary tract or any other specific type. The smaller study of Bertazzi et al. reports finding significantly increased incidence of cancer in their cohort when different types of neoplasms are grouped under fewer, less specific categories. However, the study was small, and when individuals are removed whose possible PCB exposure is so small or the latency period so short as to make any association with PCBs dubious, this study is also negative. Studies by Gustavsson and coworkers, by Nicholson and coworkers, and by Zack and Musch similarly found no statistically significant increases in cancer mortality among capacitor workers. The increase in melanoma and pancreatic cancer reported in the initial case study of very limited size by Bahn et al. was not found in any of the other mortality studies. Further, Bahn et al. neglected to consider the likely exposure to other compounds, including known carcinogens, among their group of petroleum refinery workers.

When the mortality studies are considered collectively, the epidemiological evidence for human carcinogenicity is mostly negative but still in need of the statistical power provided by larger numbers. While higher-than-expected rates have been reported in two of the smaller studies, the findings were either sex-specific or race-specific and were not supported by any of the other studies. These problems are a strong indication that the etiologic factor responsible for each increase is not the same, and such contradictory evidence is inconsistent with most of the current theories on chemical-induced carcinogenesis. In fact, one problem common to all studies to date is the failure to control for a number of important confounding risk factors. A second problem common to all studies is the absence of any relationship between the duration of exposure or of the latency period and any cause of death. Therefore, the studies to date do not provide sufficient epidemiologic evidence for cancer in humans.

1.5 CONCLUSIONS

In conclusion, administration of PCBs to animals in relatively high doses has been observed to produce a number of adverse effects. However, with the exception of dermal toxicity (chloracne), none of these effects have been convincingly demonstrated in humans from either occupational or environmental exposure to PCBs. Because specific PCB mixtures (60% chlorine) have tested positive in rodent cancer bioassays, the potential for PCBs to cause cancer is of special interest. Several epidemiologic studies have been conducted, but provide no basis upon which to conclude that PCBs are human carcinogens. Until larger epidemiological studies can be completed, that is, until a larger number of the persons included in the cohorts of these studies have died, the data must be considered inadequate to fully characterize the human carcinogenic potential of PCBs. Nevertheless, the fact that epidemiologic investigations have yet to uncover any chronic, adverse human health effect that can be causally linked to relatively high, chronic PCB exposure suggests that the relatively low level PCB exposures that occur in our daily environment pose no significant risk to the human population.

These conclusions are shared by other PCBs experts on including Dr. Renate Kimbrough of the U.S. E.P.A. In a 1987 review of the health effects of PCBs and PBB (polybrominated biphenyls), Dr. Kimbrough stated:

> *In conclusion, various toxic effects of PBBs and PCBs have been described in laboratory animals. In humans, acute poisoning outbreaks have only occurred following exposure to a combination of PCBs and PCDFs. When humans were exposed only to PCBs or PBBs, the only observed acute effects have generally been minor. So far, no significant chronic health effects have been causally associated with exposure to PCBs or PBBs.*

In a more recent review of PCBs, Dr. Kimbrough again concluded:

Several occupational studies have presented no conclusive scientific evidence that PCBs have caused cancer in humans. ... ... Thus, despite positive laboratory animal data and except for chloracne, exposure to PCBs has led to no convincing, clinically demonstrable, chronic health effects in humans.

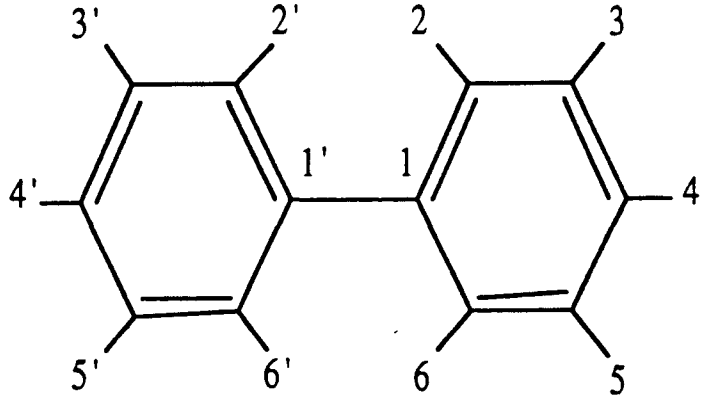
2.0 PROPERTIES OF POLYCHLORINATED BIPHENYLS (PCBs)

2.1 PHYSICAL AND CHEMICAL PROPERTIES OF PCBs

2.1.1 PCB Nomenclature and Chemical Structure

Polychlorinated biphenyls (PCBs) are a group of synthetic organic compounds which consist of a biphenyl moiety substituted with varying amounts of chlorine atoms (Figure 2.1.1). Individual PCB isomers have the empirical formula $C_{12}H_{10-n}Cl_n$ (where n = 1-10), and are formed by a number of different chemical processes, including phenylation or arylation of aromatic compounds, condensations reactions, and direct chlorination of biphenyl (Sawhney, 1986). Of these processes, only direct chlorination of biphenyl in the presence of a catalyst, such as iron filings or iron chloride, was used to prepare commercially available PCBs (Sawhney, 1986). Because of the ten possible sites of chlorination that exist on the basic biphenyl moiety, there are theoretically 209 possible isomers which can be formed; however, many of these are not likely to occur at significant levels in commercial PCB mixtures (Table 2.1.1). Indeed, Kimbrough (1980) reported that some 20 individual PCB isomers have not been found during analysis of commercial mixtures.

Individual PCB isomers are numbered as shown in Figure 2.1.1. The two carbon atoms forming the single bond between the phenyl groups are numbered 1 and 1'. The two rings are numbered identically, except that the ring which contains the carbon labeled 1' is numbered in primed numbers according to the IUPAC definitive rules. These rules are as follows: (1) an unprimed number is assigned lower order than the corresponding primed number (i.e., 2 is lower than 2'); (2) a lower number is assigned to a point of attachment in equivalent positions (i.e., 2 versus 6 for a substituent in one of the ortho positions); and (3) the ring with the greatest number of substituents is unprimed or, when the number of substituents in the two rings is the same, the unprimed numbers are assigned



Structure of

Polychlorinated Biphenyl

Figure 2.1.1

Table 2.1.1

Possible PCB Isomers

Degree of Substitution	Number of Isomers
Mono	3
Di	12
Tri	24
Tetra	42
Penta	46
Hexa	42
Hepta	24
Octa	12
Nona	3
Deca	1
Total	209

to the ring system containing substituents with the lowest-numbered chlorine not in common (Hutzinger et al., 1974; Kimbrough, 1980; Sawhney, 1986).

The nomenclature of various commercial PCB mixtures differs from that used for the individual isomers, as well as between the various commercial mixtures. PCB mixtures, along with their trade names, are identified by a numerical designation. In the United States, the most widely used commercial PCB mixtures were those manufactured by Monsanto under the trade name Aroclor®. The different Aroclors are distinguished by a four-digit number such as 1242, 1254, 1248, and 1260, etc. The first two digits in this nomenclature denote the type of molecule that has been chlorinated. A designation of 12 indicates a chlorinated biphenyl, while a 54 in the first two digits indicates a chlorinated terphenyl. Those Aroclors beginning with the numbers 25-- and 44-- are blends of PCB and chlorinated terphenyls (75% and 60% PCB, respectively) (Hutzinger et al.,

1974). The latter two digits indicate the weight percentage of chlorine in the mixture. An exception to this rule was Aroclor 1016. Despite its name, the weight percentage of chlorine in Aroclor 1016 was on average 41%, not 16% as its name implies. Aroclor 1016 was so named because the penta-, hexa-, and heptachlorobiphenyl content had been significantly reduced while retaining an average chlorine content similar to Aroclor 1242 (Hutzinger et al., 1974). In other commercial preparations the numerical code may indicate the approximate mean number of chlorine atoms per PCB molecule (Phenoclor, Clophen, Kanechlor) or simply the weight percentage of chlorine (Fenclor). A comparison of representative PCBs can be found in Table 2.1.2. The numbers appearing under the column heading "approximate weight percentage of chlorine" in Table 2.1.2 are based on a very broad weight percentage composition since all of these commercial products are mixtures of various PCB isomers and congeners (Table 2.1.3). Commercial PCB mixtures of the same average chlorine content but different manufacture (e.g., Aroclor 1242, Clophen A30 and Kanechlor 300) will vary to some degree in PCB congener content, as well as in levels of contaminants (such as polychlorinated dibenzofurans). For example, the reported congener content of Aroclor 1242 has been shown shown to vary from one report to the next (see Table 2.1.4). Comparisons between Aroclor and Kanechlor mixtures of the same average chlorine content indicate there may have been significant differences in the congener composition of these commercial mixtures (p. II-17, USEPA, 1987). Kannan et al. (1987) recently showed the amount of three toxic, coplanar, *meta-para* chlorinated PCB congeners varies among commercial PCB mixtures and that amounts present tend to be greater in Kanechlor mixtures than in Aroclor mixtures. It would appear that the European (Clophens and Phenoclors) and Japanese PCB mixtures (Kanechlors, Arcolor T-products) also contain, on average, higher amounts of polychlorinated dibenzofuran contaminants than American-made commercial PCB mixtures (Aroclors) of the same chlorine content (Brinkman and Dekok, 1980; USEPA, 1987).

Variations in the numerous commercial PCB products have in part made identification of the individual isomers present in the mixes difficult. This especially comes to light when one attempts to identify the PCB residues from environmental samples. The elution profiles obtained from the environmental samples frequently do not resemble, in any recognizable way, the profile for one of

Table 2.1.2

Comparison of Various Series of Commercial PCB Mixtures

Aroclor	Clophen	Phenoclor	Pyralene	Kanechlor	Fenclor	Ave.# Cl Molecule	Approx. Wt. % Cl	Ave. Mol. Weight
1221						1.15	21	192
1232			2000	200		2.00	32-33	221
			1500			2.50	38	
1242	A30	DP3	3000	300	42	3.00	40-42	261
1248	A40	DP4		400		4.00	48	288
1254	A50	DP5		500	54	5.00	52-54	327
1260	A60	DP6		600	64	6-6.30	60	372
1262						6.80	62	389
					70	7.70	65	
1268						8.70	68	453
1270						9.50	70	
					DK	10.00	71	

Adapted from Brinkman and DeKok, (1980)

Table 2.1.3

Average Molecular Composition (wt. %) of Some Aroclors

	Aroclor						
n[a]	1221	1232[b]	1016	1242	1248	1254	1260
0	10						
1	50	26	2	3			
2	35	29	19	13	2		
3	4	24	57	28	18	1	
4	1	15	22	30	40	11	
5				22	36	49	12
6				4	4	34	38
7						6	41
8							8
9							1

[a] n in $C_{10}H_{10-n}Cl_n$
[b] 5% unidentified

Adapted from Hutzinger et al. (1974)

Table 2.1.4

Comparison of Analysis for PCB Mixtures Containing Approximately 42 Percent Weight of Chlorine

PCB Mixture	Weight Percent of PCB					
	Cl_1	Cl_2	Cl_3	Cl_4	Cl_5	Cl_6
Aroclor 1242[a]	1	16	49	25	8	1
	3	13	28	30	22	4
		4	39	42	14	
	1	17	39	33	10	
	1	15	51	27	7	
Pyralene 3000		11	57	29	2	
Kanechlor 300		6	60	31	1	

[a]Values in individual rows represent the results of measurement of different samples or by different investigators.

Adapted from Brinkman and DeKok's 1980 summation of various literature sources

the many standard reference materials. Thus, numerous investigations concerning the detection and identification of commercial PCB mixtures have been performed. The first attempt to identify individual isomers in PCB mixtures was done by Zalkind and Belikova (1938), when they oxidized two chlorobiphenyl mixtures (containing an average of five and seven chlorine atoms per molecule, respectively) with nitric acid. This process, which resulted in the conversion of 200 grams of the mixtures to 40 grams of chlorobenzoic acids, required up to 100 hours at reflux temperature and frequent nitric acid changes. After repeated recrystallization and purification as silver salts and amides, the acids were identified by comparison with authentic samples. These early experiments correctly identified 3,4-dichlorobenzoic acid and 2,4,5-trichlorobenzoic acid from the mixture containing an average of 5 chlorine atoms per molecule and 2,4,5-trichlorobenzoic acid and 2,3,4,5-tetrachlorobenzoic acid from the heptachloro-mixture. Today, however, this elaborate process has been replaced by much more sensitive and rapid methods of detection. The use of gas chromatography/mass spectrometry (GC/MS) using capillary columns has been highly effective in investigating the detailed structure of individual components of PCB mixtures. The combined use of gas chromatography/infrared spectrometry (GC/IR) has also been successful in the quantitative and qualitative analysis of PCB mixtures. Liquid chromatography and nuclear magnetic resonance have also been employed, although to a much lesser extent.

2.1.2 Physico-Chemical Properties

PCBs are among the more stable organic compounds known to man. PCBs have been widely used in industry because of their thermal stability, resistance to acids, bases, and other chemical agents, their stability in industrial use under conditions of oxidation and hydrolysis, low water solubility, low flammability, high electrical resistivity, favorable dielectric constants, low vapor pressure at ambient temperature, and suitable viscosity-temperature relationships. Some of these physical properties have been listed for various Aroclors in Table 2.1.5. In the past, PCBs have been used as dielectric fluids (capacitors, transformers), in industrial fluids (used in hydraulic systems, gas turbines and vacuum pumps), as fire retardants, in heat transfer systems, and in plasticizers, (adhesives, textiles, surface coatings, sealants, printing, copy paper) (see Table 2.1.6). A partial list of

Table 2.1.5

Physical Characteristics of Some Aroclor Mixtures

Aroclor: Physical Characteristic	1016	1221	1232	1242	1248	1254	1260
Density at 20°C(g/cm^3)	1.37	1.18	1.26	1.38	1.44	1.54	1.62
Chlorine (approx. %)	41	21	31	42	48	54	60
Viscosity at 98.9°C	—	31-32	30-31	34-35	36-37	44-58	72-78
Flash Point (°C)	170	141-150	152-154	176-180	193-196	ntb	ntb
Fire Point (°C)	ntb	176	238	ntb	ntb	ntb	ntb
Pour Point (°C)	—	1	-35	-19	-7	10	31
Dielectric Constant (20°C)	—	5.7	—	5.8	5.6	5.0	4.3
(100°C)	—	4.6	—	4.9	4.6	4.3	3.7
Solubility in Water at 25°C (mg/l)	—	—	—	0.24	0.054	0.012	0.003
Vapor Pressure (mm Hg, 25°C)	$4x10^{-4}$	$6.7x10^{-3}$	$4.1x10^{-3}$	$4.1x10^{-4}$	$4.9x10^{-4}$	$7.7x10^{-5}$	$4.1x10^{-5}$
Log Octanol/ Water Partition Coefficient	4.4-5.6	2.8-4.1	3.2-4.5	4.1-5.6	5.8-6.1	6.0	6.1-7.1
Conversion Factors							
1 ppm=(mg/m^3)	10.05	8.21	9.50	10.9	12.2	13.4	15.4
1 mg/m^3=(ppm)	0.0948	0.122	0.105	0.0917	0.0816	0.0745	0.0651

(ntb) indicates none to boiling.
Adapted from Kimbrough (1980); U.S.E.P.A. (1985)

Table 2.1.6

Uses of PCBs Classified According to Aroclor Grade

Current Uses of PCBs	Aroclor Grade Commonly Used
Electrical capacitors	1016, 1221, 1242, 1254
Electrical transformers	1242, 1254, 1260
Vacuum pumps	1248, 1254
Gas-transmission turbines	1221, 1242
Former Uses of PCBs	
Hydraulic fluids	1232, 1242, 1248, 1254, 1260
Plasticizer in synthetic resins	1248, 1254, 1260, 1262, 1268
Adhesives	1221, 1232, 1242, 1248, 1254
Plasticizers in rubbers	1221, 1232, 1242, 1248, 1254, 1268
Heat transfer systems	1242
Wax extender	1242, 1254, 1268
Dedusting agents	1254, 1260
Pesticide extender, inks, lubricants, cutting oils	1254
Carbonless reproducing paper	1242

Adapted from Hutzinger et al., (1974)

some of the world's major producers of PCBs, the country of production, and the PCB trade name is presented in Table 2.1.7.

While most of the individual PCB isomers are solids at room temperature, commercial mixtures (particularly the Aroclors) can be mobile oils (Aroclor 1221, 1232, 1242, and 1248) or viscous liquids (Aroclor 1254). On the other hand at room temperature, Aroclors 1260 and 1262 are sticky resins, while Aroclor 1268 is an off-white powder. The fluidity of PCB mixtures arises from the fact that individual isomers form eutetic mixtures when placed together; that is, they mutually depress one another's melting points. For this reason, most PCB mixtures (with the exceptions of Aroclors 1221 and 1268) do not crystallize, but show a "pour point" below which the mixture changes into a resinous state. The pour point for a number of the Aroclors can be found in Table 2.1.5.

The physical properties of PCB isomers and commercial mixtures vary greatly depending on the degree and position of chlorine substitution. From the data presented in Table 2.1.5 for the Aroclor series, it is apparent that with increasing chlorine substitution (i.e. increasing Aroclor number), there is generally an increase in the density, flash point, fire point (for the two listed), and pour point. There is also an increase in the distillation range (boiling point) with increasing chlorine substitution (Hutzinger et al., 1974; Kimbrough, 1980; Cairns et al., 1986). On the other hand, the dielectric constant and water solubility generally vary inversely with the degree of chlorine substitution. The decrease in the dielectric constant with an increase in chlorine substitution corresponds with an increase in electrical insulating properties and is the reason why more highly chlorinated PCB mixtures such as Aroclors 1242, 1248, 1254, and 1260 were used in electrical transformers.

The Kanechlor (Table 2.1.8), Pyralene (Table 2.1.9), Clophen (Table 2.1.10) and Fenclor (Table 2.1.11) PCB mixtures generally follow similar patterns, but there are notable exceptions. One such exception is Pyralene 1500, which has an

Table 2.1.7

The World's Major PCB Producers

Producer	Country	PCB Tradename
Bayer	Germany	Clophen®
Prodelec	France	Phenoclor® Pyralene®
Kanegafuchi	Japan	Kanechlor®
Mitsubishi-Monsanto	Japan	Santotherm® Aroclor®
Caffaro	Italy	Fenclor® Apirolio®
Sovol	USSR	Sovol®
Chemko	Czechoslovakia	Delor®
Geneva Industries	U.S.A.	
Allis-Chambers Mfg. Co.	U.S.A.	Chlorextol®
Federal Pacific Electrical Co.	U.S.A.	Dykanol®
Westinghouse	U.S.A.	Inerteen®
Wagner Electrical Corp.	U.S.A.	No-Flamol®
General Electric Co.	U.S.A.	Pyranol®
Monsanto	U.S.A.	Aroclor® Pyroclor® Therminol® Santovac® Pydraul® Turbinol®

Adapted from Hutzinger et al., (1974), and Brinkman and DeKok, (1980)

elevated dielectric constant as compared to Pyralene 2000 or 3000, but is intermediate in its other properties. These mixtures appear as thick fluids, resins, or flakes. Some, such as Pyralene 1500, have been marketed with additional stabilizers while others, like Kanechlor 1000 and Apirolio 1481T, are mixtures of PCBs and other chlorinated hydrocarbons. Kanechlor 1000 is a mixture of Kanechlor KC-500 and trichlorobenzenes while Apirolio 1481T is a mixture consisting of 60% Fenclor 64 and 40% trichlorobenzenes (Note: The Fenclor series was replaced by the Apirolio series.)

Table 2.1.8

Characteristics of Kanechlor Mixtures

Kanechlor	Density (100°C)	Viscosity at 75°C (cm^2/sec)	Distillation Range (°C)	Water Solubility (ppb) 25°C
KC-200	1.23	2-3	370-360	---
KC-300	1.32	3-4	325-360	147
KC-400	1.38	5-7	340-375	42
KC-500	1.47	12-19	365-390	8
KC-600	1.55	46-87	385-420	2

Adapted from Brinkman and DeKok, (1980)

Table 2.1.9

Characteristics of Pyralene Mixtures

Property	Pyralene 2000	Pyralene 1500	Pyralene 3000
Density[a]	1.29	1.35	1.39
Viscosity[a] (cm^2/sec)	16.20	38.00	65.00
Pour point (°C)	-37	-29	-23
Fire point (°C)	284	ntb*	ntb
Dielectric constant			
at 20°C	6.0	6.4	5.9
at 100°C	4.9	5.2	4.8

[a]At 20°C
*ntb = none to boiling
Adapted from Brinkman and DeKok, (1980)

Table 2.1.10

Characteristics of Clophen Mixtures

Property	Clophen C	Cl	T64N
Density*	1.38	1.35	1.51
Viscosity* (cm^2/sec)	56	35	27
Melting point (°C)	-22	-26	-39
Fire point (°C)	ntb**	ntb	ntb
Dielectric constant			
at 20°C	5.9	6.1	4.9
at 90°C	5.0	5.1	4.2

* At 20°C
** ntb = none to boiling
Adapted from Brinkman and DeKok, (1980)

Table 2.1.11

Characteristics of Fenclor Mixtures

Property	Fenclor 42	54	64	70	DK
Chlorine content (%)	38-41	50-54	58-62	63-66	71-72
Density (at 20°C)	1.38	1.53	1.63	1.67	1.95
Flash point (°C)	335	none	none	none	---
Pour point (°C)	-19	11	34	51	---
Viscosity at 98°C (cm^2/sec)	2	4-5	13-15	24-26	---
Vapor pressure (mmHg) at 200°C	25-30	10	3-4	2	---

Adapted from Brinkman and DeKok, (1980)

Like their physical properties, the chemical properties (solubility, vaporization and vapor pressure, and lipophilicity) of PCB isomers and commercial mixtures vary depending on the degree and position of chlorine substitution. Because these properties are important determinants of PCB transport and distribution within the environment, much information about these properties has been gathered and extensive reviews have been published. These properties will be discussed below.

The solubility of PCBs in water and in organic solvents greatly influences their transport and persistence in the environment. Hutzinger et al. (1974) published an extensive list of solubilities of a number of individual chlorobiphenyls as well as of commercial preparations. A partial list can be found in Table 2.1.12. It has been demonstrated that the solubility of PCBs in water generally varies inversely with the degree of chlorination. Individual chlorobiphenyls vary in their solubility from about 6 parts per million (ppm) for 2-monochlorobiphenyl to as low as 0.007 ppm for octachlorobiphenyl (Sawhney, 1986). Decachlorobiphenyl is an exception to this rule. In spite of its higher chlorine content, decachlorobiphenyl is about twice as soluble as the octachlorobiphenyl. Interestingly, chlorine substitutions in different positions of the biphenyl moiety also affect the solubility of chlorobiphenyls with the same chlorine content. Indeed, Hutzinger et al. (1974) demonstrated that the solubility of 2,4-, 2,2'-, and 2,4'-dichlorobiphenyl were 1.40, 1.50, and 1.88 ppm, respectively, while that of 4,4'-dichlorobiphenyl was only 0.08 ppm.

Similarly, the water solubility of PCB mixtures, such as the Aroclors, generally varies inversely with the weight percentage of chlorine present in the mixture. Hutzinger et al. (1974) reported the water solubilities of various Aroclor mixtures as follows: 1242, 200 parts per billion (ppb); 1248, 100 ppb; 1254, 40 ppb; and 1260, 25 ppb. Haque et al. (1974) reported a solubility value for Aroclor 1254 of 56 ppb, which is similar to that reported by Hutzinger et al. (1974). Kimbrough (1980), however, reported solubilities of Aroclors 1242, 1248, 1254, and 1260 as 240 ppb, 52 ppb, 12 ppb, and 3 ppb, respectively. These differences may be due to

Table 2.1.12

Solubility of Selected PCBs in Water

Compound	Solubility mg/l (ppm)
2-monochlorobiphenyl	5.9
3-monochlorobiphenyl	3.5
2,4-dichlorobiphenyl	1.4
4,4'-dichlorobiphenyl	0.08
2',3,4-trichlorobiphenyl	0.078
2,2',5,5'-tetrachlorobiphenyl	0.046
2,2',3,3'-tetrachlorobiphenyl	0.034
2,2',3,4,5'-pentachlorobiphenyl	0.022
2,2',4,4',5,5'-hexachlorobiphenyl	0.0088
2,2',3,3',4,4',5,5'-octachlorobiphenyl	0.0070
decachlorobiphenyl	0.015

Adapted from Hutzinger et al. (1974)

differences in the water used (as discussed below), the temperature of the experiment, or because of differences within the Aroclor mixtures used. Because the lesser chlorinated biphenyls are generally more soluble than the more highly chlorinated biphenyls, aqueous solutions of Aroclors show greater proportions of the lower chlorinated congeners (in the dissolved state) than the nondiluted Aroclors from which the solutions are made (Sawhney, 1986).

While the previously described aqueous solubilities represent those determined experimentally, the solubilities of Aroclor mixtures in environmental samples of water can vary greatly with differing conditions. Zitko (1970) reported the solubility of Aroclor 1254 in fresh water to be in the range of 0.3 to 3 ppm, which is much higher than that reported by MacKay and Wolkoff (1973) (0.012 ppm), Haque et al. (1974) (0.056 ppm), or Kimbrough (1980) (0.012 ppm). This occurrence may be explained by the fact that environmental samples of water generally contain dissolved organic substances which could increase the concentration of PCBs in the sample (because some additional PCB will be dissolved in the organic matter) and thereby alters the apparent aqueous solubility. Differences in the organic content of various water supplies would, therefore, affect the solubility of PCBs and hence explain some of the differences in reported solubilities.

Table 2.1.13 lists the vaporization rate ($gm/cm^2/hr$ at 100°C) for several of the Aroclors. Commercial PCB mixtures have very low vapor pressures and, like their water solubility, their vapor pressure (vaporization rate) generally decreases with increasing chlorine substitution. As can be seen in Table 2.1.13, the vaporization rate decreases about 200-fold when going from Aroclor 1221 (21% chlorine by weight), which consists primarily of mono- and dichlorobiphenyls (Table 2.1.3), to Aroclor 1260 (60% chlorine by weight), which consists of more highly chlorinated PCB isomers (penta-, hexa-, hepta-, and octachlorobiphenyls) (Table 2.1.3).

The vapor pressure and vaporization rate of PCBs in environmental samples has been shown to be greatly reduced from those values determined for commercial PCB mixtures. This is thought to result from the adsorption of PCBs onto soil or sediment surfaces. Haque et al. (1974) demonstrated that the vaporization rate also depends on the surface for adsorption. They observed that

Table 2.1.13

Vaporization Rates of Six Aroclors Measured at 100°C

Aroclor	Vaporization Rate ($gm/cm^2/hr$)
1221	0.00174
1232	0.000874
1242	0.000338
1248	0.000152
1254	0.000053
1260	0.000009

Adapted from Sawhney, (1986)

about 60% of Aroclor 1254 adsorbed by Ottawa sand was lost by vaporization in a 4-week period whereas no significant loss occurred from Woodburn soil. Furthermore, the less chlorinated biphenyl congeners in the Aroclor mixture exhibited the greatest loss and the more chlorinated biphenyl congeners the least. This latter observation is consistent with the fact that the lower chlorinated PCB isomers and mixtures generally show the highest vaporization rate. Although not thoroughly understood, differences in vaporization rates from various soils are probably due to differences in soil or sediment components, such as surface area, organic matter content, and pH (Chou and Griffin, 1986).

Unlike the vaporization rate of PCBs from soil or sediment, the vaporization from aqueous solutions is much higher than one would expect based on their low vapor pressure and high molecular weight (Sawhney, 1986). The reason for this is unknown. The loss of PCBs from aqueous solutions has been described in terms of

fugacity (the tendency of a solute to escape from solution). Based on this approach, Sawhney (1986) reported that the half-life of PCBs (the time required for 50% of an original concentration to disappear) in a well-mixed 1-meter deep body of water to be approximately 10 hours. Sawhney (1986) concluded that evaporation of PCBs from contaminated river and lake water may, therefore, represent a major means of their environmental transport.

2.1.3 Summary of the Properties of PCBs

Polychlorinated biphenyls are a group of synthetic organic compounds which consist of a biphenyl moiety substituted with varying amounts of chlorine atoms. Commercial mixtures, such as the Aroclors, are composed of varying amounts of the 209 possible PCB isomers (although not all possible isomers are formed during commercial production). It is the varied composition of these commercial PCB mixtures that may give them their substantially different properties.

The physical and chemical properties of PCBs are dependent on the extent of chlorination as well as the position of the chlorine atoms on the biphenyl moiety. The effects of alterations in chlorine content in PCB isomers and mixtures differs with respect to the physico-chemical parameter examined. With increasing chlorination, there is generally an increase in the density, flash point, fire point, pour point and distillation range, as well as an increase in adsorption to surfaces. Conversely, there is generally a decrease in the water solubility, dielectric constant, and vaporization rate with increasing chlorine substitution.

3.0 ANIMAL TOXICOLOGY OF PCBs

3.1 PHARMACOKINETICS OF PCBs

3.1.1 Absorption

The vast majority of the many studies examining the pharmacokinetics of PCBs have utilized intravenous administration and therefore provide no information regarding the absorption characteristics of PCBs. The few studies which have measured absorption of PCBs indicate that there is a potential for significant entry from a variety of routes of exposure.

Albro and Fishbein (1972) found that rats fed PCB congeners in their diet excreted 10% or less of the dose in the feces. The extent of retention of the PCB dose was largely independent of the degree of chlorination for doses ranging from 5-100 mg/kg. Allen et al.(1974b) similarly found that less than 6% of a dose of the PCB mixture Aroclor 1248, when administered by gastric intubation to monkeys in doses of 1.5 or 3.0 mg/kg, was recovered in the feces within 14 days. Tanabe and coworkers observed apparent absorption efficiencies ranging from 66 to 95% for individual PCB congeners in rats, with absorption decreasing as the number of chlorines increased (Tanabe et al., 1981). Similar degrees of absorption were found for different PCBs with the same extent of chlorination. Among these various studies, accurate estimations of extent of absorption are precluded by potentially confounding factors such as intestinal metabolism and biliary recirculation of PCBs. However, these observations strongly suggest that absorption of all PCBs by the oral route is extensive.

The results of Wester et al. (1983) indicate that there is also substantial absorption of PCBs by the dermal route. The dermal absorption of radiolabelled doses of two PCB mixtures (42% chlorination and 54% chlorination) was determined in guinea pigs, and the absorption of the 42% chlorination mixture was measured in monkeys. Urinary and fecal recovery of label following dermal application of the PCB dose was expressed as a percent of the recovery measured when the same dose was administered intravenously. The dermal application site

was washed after 24 hours, so the absorption values represent the percent of dose absorbed over this time interval. They observed that 33% of the 42%-PCB dose and 56% of the 54%-PCB dose were absorbed through the skin of guinea pigs. It was found that 15-34% of a topically applied dose of the 42% PCB mixture was absorbed in monkeys. The data from monkeys may be particularly relevant to humans in that monkeys usually have similar percutaneous absorption characteristics (Wester and Maibach, 1983).

The highly lipophilic nature of the PCB compounds makes it likely that they are also easily and efficiently absorbed in the lungs. Benthe et al. (1972) administered a PCB mixture (Pydraul A200) as an aerosol to rats and observed a rapid increase in PCB concentration in the liver. The hepatic PCB levels were approximately 40 μg/g liver 15 minutes after PCB exposure, and 70 μg/g liver 2 hours after exposure. The absence of more detailed studies of PCB absorption by this route, however, precludes definitive conclusions.

3.1.2 Distribution and Elimination

The study by Busbee et al. (1985) indicates that ingested PCBs probably move into the vascular space via the lymphatic system. High concentrations of PCBs were found in chylomicrons in the thoracic duct lymph following oral administration of labelled Aroclor 1242 in beagles, and exteriorization of the lymph flow prevented ingested PCBs from reaching the systemic circulation. In intact dogs, a significant fraction of the PCBs in blood (31%) was found in chylomicrons 30 minutes after ingestion, but this fraction diminished relatively rapidly. These observations suggest that intestinal epithelial cells either synthesize ingested PCBs into chylomicrons or sequester them within pre-formed chylomicrons, and then secrete these PCB-containing chylomicrons into the intestinal lymphatic drainage. The absorbed PCBs appear to gain access to the vascular space via the thoracic lymph duct, and are probably released from the chylomicrons during hepatic chylomicron clearance.

Once in the blood, PCBs are rapidly transported to all tissues. Because of their extremely limited aqueous solubility, PCBs in the blood are transported in

association with lipoproteins, other plasma proteins such as albumin, cellular elements of the blood, or as discussed above, with chylomicrons.

In vitro studies of the partitioning of PCBs in blood indicate that for most PCB congeners, about 20-30% of the PCBs in blood are associated with cellular elements (Vomachka et al., 1983; Matthews et al., 1984). Estimates of the percentage of blood-borne PCBs distributed among the various lipoprotein fractions vary somewhat. The results from three studies of *in vitro* partitioning of 2,4,5,2',4',5'-hexachlorobiphenyl in plasma lipoprotein fractions are summarized in Table 3.1.1. The 1983 study by Vomachka et al. found the hexachlorobiphenyl to

Table 3.1.1

Plasma Distribution of 2,4,5,2',4',5'-Hexachlorobiphenyl

	Percent Plasma PCB/Fraction				
	VLDL	LDL	HDL	BF[a]	Reference
rat	30%	17%	18%	32%	Vomachka et al. (1983)
human	12%	57%	31%	ND[b]	Maliwal & Guthrie (1982)
	23%	19%	27%	31%	Vomachka et al. (1983)

[a] BF is bottom fraction and contains PCB bound to albumin and steroid-binding globulin.
[b] Not Determined. Percentages are for lipoproteins only.

be roughly evenly distributed among the fractions in both rats and humans, while the Maliwal and Guthrie (1982) study found that substantially more PCB was distributed to the LDL fraction than the others. [There are *in vitro* lipoprotein distribution data in the report by Gallenberg and Vodicnik (1987 a&b), but it appears to be the same data presented in the Vomachka et al. paper]. There is agreement that PCB molecules can be rapidly exchanged among lipoproteins, and that the distribution does not seem to be affected by PCB concentration (Vomachka

et al., 1983; Maliwal & Guthrie, 1982; Matthews et al., 1984; Gallenberg and Vodnicnik, 1987a&b).

Further insight into the fate of PCBs in the blood can be gained from *in vivo* experiments examining the partitioning of PCBs and their metabolites among various blood fractions. Busbee et al. (1985) examined the distribution of orally-administered, radiolabelled Aroclor 1242 in beagle dogs. They found that the highest percentages of PCB-label associated with chylomicrons or lipoproteins were at the earliest time points examined (31% in chylomicrons at 30 min.; 10% or less each for VLDL and LDL at 1 hr). The percentages in these fractions decreased with time, while the percentage associated with albumin increased from 52% at 30 minutes to 78% at 2 hours and remained relatively constant. Matthews et al. (1984) reported similar observations in rats administered 2,4,5,2',5'-pentachlorobiphenyl (^{14}C-labelled) intravenously. At the earliest time point (10 min.), most of the radioactivity was found in the combined lipoprotein fractions. However, the percentages bound to lipoproteins declined with time, and the percentage associated with other plasma proteins increased from 40% at 10 minutes to over 80% at 8 hours.

Although the percent of radioactivity distributed to lipoproteins declined with time in these experiments, this decline probably is not due to a change in affinity of the PCB for lipoproteins. Gallenberg and Vodicnik (1987a&b), when examining PCB distribution during the substantial lipoprotein changes associated with pregnancy in rats, found that the affinity of their hexachlorobiphenyl for individual lipoprotein fractions did not appear to change. A more likely explanation for the decline in the percentage of radioactivity associated with the lipoprotein fractions in the Busbee et al. and Matthews et al. experiments appears to be related to the metabolism of PCBs. In the study of Matthews et al. (1984), it was found that the primary metabolite of 2,4,5,2',5'- pentachlorobiphenyl, the 3-hydroxy- derivative, is bound almost exclusively to albumin. It is reasonable to assume that with time, the percentage of radiolabel in the blood representing the parent PCB molecule declines due to distribution to tissues and metabolism, while the percentage of label representing the metabolite increases. Since the parent PCB molecule is primarily associated with lipoprotein and the metabolite is

associated with albumin, the percentage of total radiolabel partitioned to lipoproteins will decrease with time while that partitioned to albumin will increase. Matthews et al. (1984) indicated that in a previous study with 2,4,5,2',5'-pentachlorobiphenyl, approximately 80% of the total PCBs in the blood at 8 hours was in the form of metabolites (Matthews and Anderson, 1975a&b). This corresponds closely with the approximate 80% of radiolabel associated with albumin at this time point.

The nature of the distribution of PCBs to various tissues and their elimination has been the subject of a number of investigations. Matthews and Anderson (1975b) examined the effect of chlorination on the distribution and elimination of PCBs after giving rats a single dose of radiolabeled mono-, di-, penta- or hexachlorobiphenyl. After injecting 600 μg/kg of the PCB intravenously, the authors found that better than 90 percent of the dose was cleared from the blood within the first 15 minutes. The dominant factors influencing the initial distribution of the PCBs were found to be tissue volume, tissue/blood partition ratios, adsorption to proteins, and perfusion rate. PCBs were initially taken up by liver, a tissue with a high perfusion rate and moderate affinity for PCBs, and muscle, a tissue which also has a high perfusion rate and constitutes a large percentage of total body mass. Following the rapid initial distribution there was a redistribution influenced primarily by the lipophilicity of the PCBs. This redistribution resulted an an accumulation of the PCBs and their metabolites in skin and adipose tissues, both tissues characterized by the highest affinity for PCBs but low perfusion rates. Similar distribution and redistribution patterns were reported by Tuey and Matthews (1977), who examined the distribution and elimination of a 600 μg/kg dose of 3,3',5,5'- tetrachlorobiphenyl in the rat, and Muhlebach and Bickel (1981) and Muhlebach et al. (1985) who studied 2,4,5,2',4',5'-hexachlorobiphenyl disposition in rats.

In view of the highly lipophilic nature of the PCBs, it is not surprising that they ultimately distribute to adipose tissue. Factors which influence the extent to which PCBs accumulate in fat logically include both the affinity of the PCB for adipose tissue and its rate of metabolism and elimination. The affinity of PCBs for adipose tissue is a function of their lipid solubility, and solubility can vary with

both the extent and position of chlorination of the PCB molecule (Matthews et al., 1984). Metabolism can also be influenced by the chlorination of the PCB. This is discussed in some detail in Section 3.1.3, Metabolism.

There is considerable experimental evidence supporting the concept that the extent and position of chlorination of the PCB molecule influence its distribution and elimination. For example, Gage and Holm (1976) studied the retention of a variety of PCB congeners in the fat of mice, and found that the accumulation was dependent upon both the number of chlorines per molecule and their positions. Similarly, Benthe and Schmoldt (1973) injected rats with 1,000 mg/kg of a PCB mixture primarily composed of tetrachlorobiphenyls and measured the specific isomer profile in fat tissue 4 weeks later. The proportion of penta- and hexachlorobiphenyls had changed from 5% in the injected PCB mixture to 63% of the fat PCB content. Benthe and Schmoldt (1973) concluded that the decreased proportion of tri- and tetra- chlorobiphenyls in rat adipose tissue suggests a higher metabolism and elimination of these less-chlorinated PCB isomers. The importance of the degree of chlorination upon elimination is further demonstrated by the study of Matthews and Anderson (1975b), who measured distribution and elimination of PCB congeners with from one to six chlorines. The estimated terminal elimination half-lives measured for the various congeners were: 15.7 hours for 4- chlorobiphenyl, 22.2 hours for 4,4'- dichlorobiphenyl, 211 hours for 2,4,5,2',5'- pentachlorobiphenyl, and 642 hours for 2,4,5,2',4',5'-hexachlorobiphenyl. To a limited extent, there are indirect observations which indicate a similar influence of extent of chlorination on PCB elimination in man. For example, Chen and Hites (1983) found that two years after exposure to a high dose of a PCB mixture, almost all of the congeners with 5 or fewer chlorines had been eliminated. PCBs with 6 or more chlorines appeared to have been retained.

The importance of the position of chlorination in influencing the elimination of PCBs has been demonstrated in a number of studies. For example, Kato et al. (1980) determined the rate of elimination of four different symmetrical hexachlorobiphenyl congeners in rats. Despite equivalent chlorination among these congeners, the percent of dose excreted over a seven-day period ranged from

4.49% of the dose for 2,3,5,2',3',5'- hexachlorobiphenyl to 92% for 2,3,6,2',3',6'-hexachlorobiphenyl. Birnbaum (1983) used two hexachlorobiphenyl congeners to demonstrate the importance of the position of chlorination for elimination of PCBs in the senescent rat. In this study, she found that more than 50% of an intravenous 600 μg/kg dose of 2,3,6,2',3',6'-hexachlorobiphenyl was metabolized and excreted via the bile into the feces within two days. In contrast, the 2,4,5,2',4',5'-hexachlorobiphenyl congener redistributed to the adipose tissue from the liver, skin, and muscle, and only 2% was excreted in 21 days. Mitzutani et al. (1977; 1980) determined the disposition of five different tetrachlorobiphenyls and six different hexachlorobiphenyls in female mice. Elimination half- lives varied from one to nine days for the tetrachlorobiphenyl congeners and from 3.2 to 94 days for the hexachlorobiphenyls. In view of these ranges in elimination rates for both tetra- and hexachloro- congeners, it is not surprising that they found widely different PCB body burdens after 20 days of exposure to congeners of equivalent chlorination. The extent of influence of position of chlorination on PCB elimination may vary with species. For example, the comparative accumulations of various *ortho* chloro-substituted hexachlorobiphenyls were found to differ among rats, rabbits, quail, and trout (Sparling and Safe, 1980a&b).

A number of investigators have reported significant differences among species in distribution and elimination rates of PCBs. For example, in a study by Sipes et al. (1980) the disposition of a 600 μg/kg intravenous dose of 4,4'-dichlorobiphenyl was studied in the beagle dog and the cynomolgus monkey (*Macaca fascicularis)*. The biological half-life of 4,4'-dichlorobiphenyl was 33 hours in the dog, with extensive biliary excretion of metabolites. In the monkey, however, the biological half-life was approximately 19 days, and the PCB metabolites were excreted mainly in the urine.

These dog-monkey comparisons were subsequently extended by this group to include the 2,3,6,2',3',6'-hexachlorobiphenyl compound (Sipes et al., 1982a). As in their previous study, they found an initial rapid distribution of the PCB to the liver and muscle, followed by metabolism and elimination, or a redistribution of the parent compound into adipose tissue. Differences in elimination between dog and monkey were less pronounced for the hexachlorobiphenyl than that previously

observed for the dichlorobiphenyl. The terminal elimination half-life was approximately 3 days for the dog and 4.6 days for the monkey. Biliary excretion was the primary elimination route in both species. Dog and monkey pharmacokinetic comparisons were also made by this group (Sipes et al., 1982b) for another hexachlorobiphenyl, 2,4,5,2',4',5'-hexachlorobiphenyl. This study not only demonstrates species differences in PCB disposition, but when compared with the results obtained by this group with the 2,3,6,2',3',6'-hexachlorobiphenyl, provides further illustration of the importance of the position of chlorination for PCB elimination. PCB elimination half-lives were substantially different between monkey and dog for the 2,4,5,2',4',5'-hexachlorobiphenyl (15.4 days for the dog and 46.2 days for the monkey) and between hexachlorobiphenyls for each species with the 2,3,6 isomer having a shorter half-life in both dog and monkey. The vast majority of the dose of 2,4,5,2',4',5'-hexachlorobiphenyl was excreted in the bile in both species.

The monkey may also have a slower elimination rate for PCBs than the rat, as indicated by the study of Abdel-Hamid et al. (1981). In this study, female rhesus monkeys eliminated an intravenous dose (600 µg/kg) of 3,4,3',4'-tetrachlorobiphenyl more slowly than either male or female rats given the same dose. The fecal route of elimination was predominant in both species. There did not appear to be a sex difference in PCB elimination, as male and female rats showed similar tissue accumulation and elimination rates.

Evidence has been presented which suggests that PCB distribution both within and among fatty tissues may not be homogenous. Ryerson et al. (1984) determined the adipose tissue levels of PCBs (parent compound and metabolite) after administration of a single i.v. dose (600 µg/kg) of 2,4,5,2',4',5'-hexachlorobiphenyl to dogs and monkeys. Pericardial, perirenal, peritesticular, and subcutaneous fat, as well as omentum, were sampled. Although PCB concentrations were similar among tissues at early and late time points, significant variation among fatty tissues was encountered near the times of peak concentration (approximately one day in the dog and four days in the monkey). These authors also report that up to two-fold variability may be encountered with samples taken from a single adipose tissue in a given animal,

suggesting that distribution of PCBs within a fatty tissue may not be homogenous.

Colburn and Matthews (1979) have also suggested a heterogenous distribution of PCBs in adipose tissue based upon a different type of observation. Rats were administered a single i.v. dose of ^{14}C-labelled 2,4,5,2',4',5'-hexachlorobiphenyl (2 μmol/kg) followed 14 days later by another single i.v. dose of equal size of the hexachlorobiphenyl, this time labelled with tritium. By using a dual-label technique the fate of the two doses could be followed simultaneously. They found that the distribution and excretion rates for the two doses were not the same, and that the second dose was distributed and excreted more rapidly than the first. Based upon this observation, Colburn and Matthews postulated that successive doses of PCBs were not distributed homogenously in fat tissue, and that a larger fraction of the most recent dose would be nearer the adipocyte cell surface where it could more readily equilibrate with the blood and be eliminated. This phenomenon was referred to as "last-in, first-out." Though an interesting concept, the relevance of the "last-in, first-out" phenomenon to PCB disposition is unclear in view of the recent study of Gallenberg and Vodicnik (1987b). These authors also administered two successive doses of 2,4,5,2',4',5'-hexachlorobiphenyl to rats and found no differences in distribution. The reason for the discrepancy between their results and those of Colburn and Matthews is unclear, as both used animals of the same strain, sex, and approximate age, and the study designs employed the same dose and time course. The Gallenberg and Vodicnik study did not use hexachlorobiphenyls with two labels, but rather used one labelled and one unlabelled dose. Though somewhat less elegant and perhaps less sensitive, the Gallenberg and Vodicnik design would be expected nonetheless to yield the same fundamental observations as the dual-label technique of Colburn and Matthews. Until this discrepancy is resolved, it is difficult to say whether PCBs exhibit the "last-in, first-out" phenomenon.

Though PCBs may be metabolized, it is the parent compound which preferentially accumulates in fatty tissues (Gage and Holm, 1976; Berlin et al., 1975), and most of the body burden of PCBs is typically found in fat. Conditions which result in fat mobilization cause a redistribution of PCBs, as indicated in the study of Wyss et al. (1982). In this study, approximately 75% of a single dose of

2,4,5,2',4',5'-hexachlorobiphenyl was retained in the fat of male rats. This body burden remained essentially constant over 9 months indicating no elimination of the compound. Some rats were fed a caloric-restricted diet which caused a decline in body and tissue weights (except skin and brain) over a four-week period. During this period, the concentration of PCBs in skin, muscle, brain, liver, lung, and blood increased. The amount of adipose tissue and the PCB concentrations in adipose tissue both decreased. After the fourth week on the restricted diet, concentrations in all tissues except the skin decreased in an apparent first-order fashion. Skin PCB concentrations remained relatively constant through the remainder of the experiment. While the rats were fed the restricted diet, their PCB excretion was increased 10-fold, and nearly all of the excreted PCB was parent compound. Approximately half of the PCB lost from adipose tissue storage during the restricted diet was excreted and half was translocated to skin. The observation that the adipose tissue PCB concentration declined indicates that loss of PCB from fat is not due to saturation, and may reflect non-homogeneity in adipose distribution or an alteration in the structural basis for PCB storage in fat.

Other conditions besides dietary restriction may cause mobilization of PCBs from their distribution sites in adipose tissue. Kurachi and Mio (1983b) found a redistribution of PCBs in mice fed Kanechlor 400 from adipose tissue to the li· when the mice were forced to walk continuously for up to three days. Nakagawa and coworkers found that treatment of rats with the hypolipidemic drug clofibrate resulted in substantial decreases in adipose tissue burdens of PCBs (Nakagawa et al., 1986). Perhaps of more importance are the studies which indicate substantial mobilization of PCB stores and their excretion in breast milk. Vodicnik and coworkers (Vodicnik et al. 1980; Vodicnik and Lech, 1980) have reported that lactating mice administered 2,4,5,2',4',5'-hexachlorobiphenyl can transfer most of their body burden to their nursing offspring within a period of twenty days. By comparison, the PCB body burden of virgin (non-lactating) mice was essentially unchanged. Translocation of PCBs from adipose tissue stores to the mammary gland may be accomplished through association with VLDL, which is substantially elevated during late pregnancy and lactation (Gallenberg and Vodicnik, 1987a). Elevated lipoprotein lipase in the lactating mammary gland breaks down VLDL to derive fatty acids for milk production, perhaps causing a

disassociation of the PCB. This would explain the observation that when hexachlorobiphenyl associated with VLDL was administered to pregnant mice there was a preferential distribution of the PCB to the mammary gland as opposed to other adipose tissue (Gallenberg and Vodicnik, 1987a).

Yoshimura and Yamamoto (1975) observed that rats administered an intravenous dose of 2,4,3',4'-tetrachlorobiphenyl excreted a portion of the dose in the feces, even when the bile duct was ligated. All of the PCB in the feces was in the form of the parent compound. Examination of the gut contents suggested that this movement of PCB into the gut occurs in the small intestine. While excretion of the PCB into the intestine by a non-biliary pathway represents a potential new route of elimination, the quantitative importance of this route is as yet unclear.

It is apparent from the literature that both the extent and position of chlorination can profoundly influence the distribution and elimination of PCB congeners, and that there can be important species differences in the disposition of PCBs. These principles imply that when exposure is to a mixture of PCB congeners (as is nearly always the case with environmental exposure), the quantitative relationships among individual congeners in the body will change with time. Those congeners that are difficult to eliminate will become predominant in the body, because easier-to-eliminate congeners will constitute progressively smaller percentages of the total body burden. After a period of time, the pattern of isomers in tissues may no longer resemble that of the mixture to which the subject was exposed, and the changes in pattern may vary from tissue to tissue and from species to species. Perhaps one of the studies which best illustrates this is the one by Hansen et al. (1983). Chickens and swine were fed Aroclor 1254. One group of chickens was fed fat (rendered into lard and incorporated into the diet) from the Aroclor 1254-fed swine. Concentrations of 18 congeners were determined in the Aroclor mixture, the fat of swine fed the mixture, the fat and liver of chickens fed the mixture, and the fat and liver of chickens fed fat from the Aroclor-exposed swine. None of the congener patterns were the same.

3.1.3 Metabolism in Animals

Metabolism of PCBs has a significant effect on their disposition. Compounds with the lipophilicity of PCBs are in general very difficult to eliminate. While some elimination from passive diffusion processes can occur, the majority of the body burden of PCBs can be eliminated only after conversion to a more polar form. The importance of the metabolism of PCBs in their disposition has stimulated a considerable amount of research in this area. This document will attempt only to summarize the most significant of these reports.

PCBs are classified according to the scheme of Matthews and Kato (1979) as Type III compounds, viz. aromatic hydrocarbons with only halogenated substitution. Like that of other Type III compounds, the metabolism of PCBs consists primarily of hydroxylation at one or more positions, often followed by conjugation.

The rate of hydroxylation of PCBs depends upon the degree of chlorination, and as a general rule the less-chlorinated congeners are more readily metabolized than are the more highly-chlorinated congeners (Hutzinger et al., 1972b; Benthe and Schmoldt 1973; Goto et al., 1974a&b; Matthews and Anderson, 1975b; Goto et al., 1975; Greb et al., 1975; Sugiura et al., 1975; Gardner et al., 1976; Peterson et al., 1976; Sundstrom et al., 1976; Tuey and Matthews, 1977; Matthews and Dedrick, 1984). A chlorine at any position in the ring will tend to reduce the rate of metabolism at that position, as non-chlorinated carbons are more readily hydroxylated. Once the biphenyl nucleus is completely chlorinated, as in the case of decachlorobiphenyl, the rat no longer metabolizes it (Goto et al., 1975). Because for the most part PCBs must be metabolized in order to be eliminated, it is not surprising to find that some of the more highly-chlorinated congeners of PCBs persist in tissues for a considerable time after exposure has ceased (Burse et al., 1974; Benthe and Schmoldt, 1973).

The rate of hydroxylation is also profoundly influenced by the positions of chlorination. A knowledge of the mechanisms of hydroxylation of the biphenyl molecule is crucial in understanding the importance of the sites of chlorination.

Although biphenyl is metabolized in the *ortho*-, *meta*-, and *para*-positions, the linear nature of the biphenyl molecule tends to direct metabolism towards its ends. The primary metabolite of biphenyl is therefore the para-hydroxylated metabolite, 4-hydroxybiphenyl (Billings and McMahon, 1978; Matthews and Dedrick, 1984). Cytochrome P-450 isozymes (e.g., cytochrome P-450 enzyme subpopulations with differing substrate and spectral properties) are responsible for hydroxylation of the biphenyl nucleus of PCBs. Cytochrome P-450 isozymes are classically divided into two groups, cytochrome P-450 and cytochrome P-448. Analyses of cytochrome P-450 isozymes suggest that cytochrome P-450 produces the 4-hydroxy metabolite, while cytochrome P-448 is responsible for the small amounts of 2-hydroxybiphenyl generated (Matthews and Dedrick, 1984). While 3-hydroxybiphenyl formation appears to result from a direct insertion reaction (Billings and McMahon, 1978), 2- and 4-hydroxybiphenyl are both believed to arise from arene oxide intermediates.

The first evidence supporting the concept that arene oxide intermediates are generated during the formation of PCB hydroxylated metabolites was obtained by Gardner et al. (1973) who isolated a dihydrodihydroxy- metabolite from 2,5,2',5'-tetrachlorobiphenyl. This metabolite was probably generated by hydration of an epoxide rather than two subsequent hydroxylations at adjacent carbon atoms. In studies similar to earlier work by Daly et al. (1972), Safe and coworkers (Safe et al., 1975; Safe and Jones, 1976; Wyndham and Safe, 1978) demonstrated that an NIH shift occurred in the formation of 4'-hydroxy metabolites of 4-chlorobiphenyl and 4,4'-dichlorobiphenyl. These results are consistent with the formation of an arene oxide intermediate.

The requirement for two adjacent unsubstituted carbons for arene oxide formation, coupled with the role of arene oxide formation in the hydroxylation of two of the three possible hydroxylation sites on a biphenyl ring, makes it clear why the position of chlorination is at least as important as the extent of chlorination in influencing the metabolic rate. For example, substitution in the *para* or 4 and 4' positions affects the favored route of metabolism (Tuey and Matthews, 1977; Matthews and Dedrick, 1984). In addition, when vicinal or adjacent carbons in the ring are chlorinated, the rate of metabolism is reduced further as the preferred mechanism of arene oxide formation is inhibited because both carbons to be

involved in epoxide formation are already bonded to chlorines. Thus, at least two adjacent unsubstituted ring carbons are required, particularly in positions 3,4,5 or 3',4',5', for the rapid metabolism of PCBs (Tuey and Matthews, 1977; USEPA, 1980; Drill et al., 1982; Matthews and Dedrick, 1984). Conversely, PCB isomers chlorinated in the 3,4,5 and 3',4',5' positions tend to be metabolized slowly and are therefore more likely to bioaccumulate. The conclusion that rate of metabolism is the major determinant of the degree of bioaccumulation is strongly supported by the distribution and elimination studies previously discussed (see Section 3.1.2).

These principles of structural requirements for metabolism developed in animal studies also appear to apply in man. Jensen and Sundstrom (1974), in analyzing the PCB congener content of human adipose tissue, concluded that two adjacent unsubstituted carbon atoms were required in order for the PCB congener to be easily hydroxylated. Similarly, Chen et al. (1982) followed the elimination of PCB congeners in individuals exposed to high levels and found that tetra- and pentachlorobiphenyls with *meta-* and *para-* unsubstituted carbons were eliminated rapidly, while those with unsubstituted *ortho-* and *meta-* carbons were eliminated more slowly.

Arene oxide metabolites, in addition to forming hydroxylated derivatives, may also give rise to methylthio- and methylsulfonyl- metabolites through conjugation with glutathione (Preston et al., 1984; Kurachi and Mio, 1983b; Kurachi, 1983; Klasson-Wehler et al., 1987). Participation of gut microbial flora is required, cleaving the degradation products of the glutathione conjugates (cysteine conjugates and mercapturic acids) appearing in the gut from the bile into their corresponding thiols (Bergman et al., 1982; Brandt et al., 1982a; Bakke et al., 1983; Preston et al., 1984). Following methylation and reabsorption of these thiols, they are further oxidized to methylsulfones and distributed by the blood. Methylthio- derivatives of PCBs have been found following PCB exposure in mice, rats, seals, and humans (Mio et al., 1976; Bakke et al., 1982; Jensen and Jansson, 1976; Yoshida and Nakamura, 1978; Haraguchi et al., 1984). The position and number of chlorines on the PCB molecule are important in determining the extent of this pathway of metabolism (Bergman et al., 1979). For some congeners, e.g. 2,4',5-trichlorobiphenyl, this pathway may be as extensive as hydroxylation

pathways (Bakke et al., 1983).

In contrast to the hydroxylated metabolites, the methylsulfonyl derivatives of PCBs may be persistent in tissues. They are capable of binding to specific cytosolic proteins in tissues, especially in the lung (Brandt and Bergman, 1981; Brandt et al., 1982b; Bergman et al., 1979; Klasson-Wehler et al., 1987). Lund et al. (1986a&b) found that tritiated 4,4'-bis(methyl-sulphonyl)- 2,2',5,5'-tetrachlorobiphenyl (MS-TCB) exhibited a high affinity and relatively high capacity for binding to cytosolic protein from rat lung. This binding was reversible, as indicated by experiments showing dissociation of label in the presence of added unlabelled MS-TCB. Lower levels of binding could also be demonstrated for cytosolic protein fractions from prostate, kidney, and large intestine. The binding protein was characterized, and was found in lung lavage fluid from mice, rats, and humans. When mice were administered an i.v. dose of MS-TCB, approximately 33% of the dose could be recovered by lavaging the lungs. Binding of methylsulfonyl metabolites to a secreted protein in the lungs is consistent with the observation that in the preferential accumulation of these derivatives in the lung, they are associated primarily with Clara cells which are considered to be secretory (Lund et al., 1985; Brandt et al., 1985). Though the toxicological significance of this accumulation in the lungs in unclear, there is evidence that methylsulfonyl metabolites may inhibit cytochrome P-450 dependent metabolism in the mouse lung (Lund et al., 1986c).

Additional metabolites of PCBs include biphenyldiol and dihydrodihydroxybiphenyl derivatives, as well as biphenyltriols, methoxy derivatives, and dechlorinated metabolites (Sundstrom et al., 1976; Gardner et al., 1973; Hsu et al., 1975; Berlin et al., 1975; Chen et al., 1976; Lucier et al., 1978; Milling et al., 1979; Safe, 1984). Hydroxylated metabolites are readily conjugated and with few exceptions do not accumulate. It has been suggested that a deficiency in glucuronidation may lead to an inability to eliminate hydroxylated metabolites and an increased susceptibility to PCB toxicity (Calabrese, 1977), though this has never been demonstrated.

The overall mechanisms of metabolism for PCBs appear to be similar for most species, but the capacity to metabolize PCBs varies widely. The rate of metabolism in animals declines in the following manner: mammals > birds > fish (Hutzinger et al., 1972b). Among mammalian species the order is dogs > rats and mice > monkeys (Matthews and Dedrick, 1984). *In vitro* studies with human liver microsomal suspensions suggest that the metabolism of hexachlorobiphenyl isomers may be slower than for other mammalian species (Schnellman et al., 1983). Metabolism must be occurring *in vivo*, however, as analysis of human tissue reveals a relative absence of those PCB forms which would most easily be metabolized (i.e. those having adjacent unsubstituted carbon atoms).

While metabolism is clearly very important in the elimination of PCBs, it has been proposed that the metabolism of certain PCB congeners may increase their toxicity. Yamamoto and Yoshimura (1973) determined the metabolism of 2,4,3',4'-tetrachlorobiphenyl in rats and discovered that the major metabolite was the 5-hydroxy derivative. They subsequently compared the toxicity of the 5-hydroxy metabolite with the parent compound in mice and found that the metabolite five times more toxic (based upon 96 hr lethality studies: LD_{50} of parent tetrachlorobiphenyl, 2.15 g/kg; LD_{50} of the 5-hydroxy metabolite, 0.43 g/kg). The position of chlorination would seem to influence toxic metabolite formation, as the major metabolites of another tetrachlorobiphenyl, the 5-hydroxy and 4-hydroxy derivatives of 3,4,3',4'-tetrachlorobiphenyl, were each <u>less</u> toxic than the parent compound as determined by liver hypertrophy and thymic atrophy (Yoshimura et al., 1987). Although in the case of 2,4,3',4'-tetrachlorobiphenyl the hydroxylated metabolite is more toxic on a molar basis, it is unclear to what extent this metabolite contributes to toxicity *in vivo*. As is the case with other PCB congeners, the hydroxylated metabolite would be expected to be excreted much more rapidly than the parent compound. This metabolite may, therefore, not achieve concentrations high enough after exposure to 2,4,3',4'-tetrachlorobiphenyl to contribute substantially to its toxicity.

In some cases, PCB metabolites may be more toxic than their parent congeners because of increased chemical reactivity. Shimada has published a series of studies which indicate that hepatic microsomal metabolism of some PCB

congeners results in covalent binding to proteins both *in vitro* and *in vivo* (Shimada, 1976; Shimada and Sato, 1978). Both phenobarbital and 3-methylcholanthrene inducible forms of cytochrome P-450 appear to participate in this type of reaction, depending upon the position of chlorination of the PCB congener (Shimada and Sawabe, 1983, Table 3.1.2). (A more complete discussion of the possible relationship between metabolite formation and toxicity is found in Section 3.3, Mechanisms of Toxicity.)

Binding of metabolite(s) from 3,4,3',4'-tetrachlorobiphenyl during *in vitro* studies was inhibited by the presence of any of a variety of sulfhydryl-containing compounds (e.g. glutathione, cysteine, etc.), and the covalent binding occurred preferentially to proteins which contain a free sulfhydryl. Shimada and Sawabe (1983) also observed destruction of the heme of cytochrome P-448 during the microsomal metabolism of 3,4,3',4'-tetrachlorobiphenyl, and addition of erythrocytes to a reconstituted monooxygenase system caused a shift in the covalent binding of 3,4,3',4'-tetrachlorobiphenyl metabolite(s) from the monooxygenase proteins to the hemoglobin of the erythrocytes (Shimada et al., 1985).

Table 3.1.2

Tetrachlorobiphenyl (TCB)-Binding Activity of Liver Microsomes Prepared from Control, Phenobarbital-, and Methylcholanthrene-Pretreated Rats

	TCB-binding activity (pmol/mg protein/min)		
Treatment	2,4,2',4'-TCB	2,4,2',5'-TCB	3,4,3',4'-TCB
none	7.13 ± 1.11	11.1 ± 2.02	0.42 ± 0.08
phenobarbital	57.9 ± 8.46*	289. ± 60.0*	0.41 ± 0.12
methylcholanthrene	5.95 ± 1.10	9.2 ± 2.61	19.9 ± 4.00*

* significantly different from corresponding control.
Adapted from Shimada and Sawabe, (1983)

3.1.4 Pharmacokinetic Modeling of PCB Tissue Concentrations

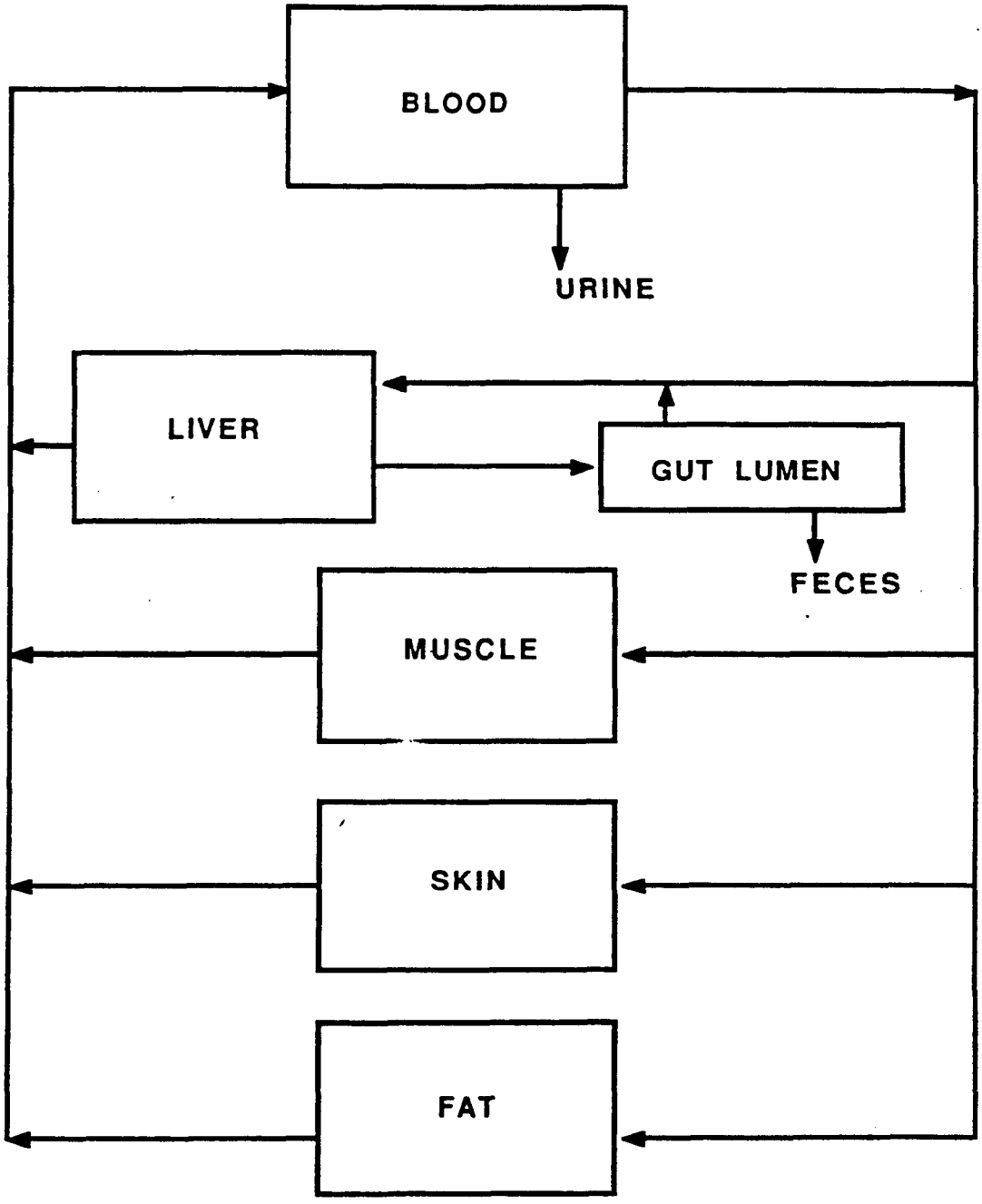
Attempts to provide a quantitative description of the distribution and elimination of PCBs have typically been limited to expression of the percent of a radiolabelled dose present in a tissue or excreted over a period of time, or as an estimate of half life. A more definitive description of PCB disposition in pharmacokinetic terms can be an imposing task in that there are both distribution and redistribution phases, and the toxicological effects of PCBs may alter both the clearance and volume of distribution with time. The difficulty in deriving useful descriptive terms is further compounded when the exposure is to a mixture of PCBs rather than a single congener.

Some investigators have attempted to apply physiological pharmacokinetic modelling techniques to PCBs (Lutz et al., 1977; Anderson et al., 1977; Tuey and Matthews, 1977; Bungay et al., 1979). In this approach, each tissue is considered to be a separate physiological compartment into which the PCB may distribute and, in some cases, be metabolized. The body is viewed as a collection of these compartments, each of which is in constant communication with the blood supply. A flow diagram of this pharmacokinetic model as proposed by Anderson et al. (1977) is depicted in Figure 3.1.1. From a quantitative standpoint, the model is made up of a set of partial differential equations that describe the mass balances of the chemical species (PCB and metabolites) in each tissue compartment. The review by Matthews and Dedrick (1984) presents a general form for the mass balance equation for a tissue which metabolizes PCBs:

$$d(V_L C_L)/dt = Q_L(C_B-(C_L/R_L)) - K_m(C_L/R_L)$$

where
- t = time
- V = volume of the tissue
- C = concentration of chemical species
- Q = blood flow rate for that tissue
- K_m = metabolic clearance
- R = equilibrium tissue-to-blood distribution ratio

Note: In the above equation the subscripts L and B denote the liver and blood, respectively.



Physiological Pharmacokinetic Model for PCBs

Figure 3.1.1

For a compartment in which there is no metabolism or in which the rate is so small that it may be considered negligible (e.g. adipose tissue, [A]), the mass balance takes the form:

$$d(V_A C_A)/dt = Q_A((C_B - (C_A/R_A))$$

In the preceding equations the authors note that the product of the tissue volume and tissue concentration equals the amount of PCB in the tissue and was written in the derivative form to allow for the fact that the volume may not be constant on a time scale relevant to the pharmacokinetics of very slowly-cleared PCBs. Another important concept inherent to these two equations is flow limitation, i.e. it is assumed that the PCB in the blood leaving any tissue is in equilibrium with that tissue. This assumption has not been explored thoroughly, but the pharmacokinetic simulations based on this assumption have been satisfactory with the exception of the skin, a tissue for which the intercompartment transport parameter has to be reduced by ten (Matthews and Dedrick, 1984).

The pharmacokinetic model can easily be applied to a number of different isomers of PCBs because the compartment sizes and tissue blood flow rates in the rat are of course independent of the chemical used. Thus, only the metabolic clearance for those tissues involved with this process needs to be measured or estimated prior to modeling the fate of a particular PCB isomer in the rat. Similarly, most of the needed parameters concerning the various tissue compartments can be generated for other test species and man. But the most important of these, the K_m which represents the metabolic clearance, is very species-dependent. In the case of man this would probably have to be predicted from an *in vitro* method (Matthews and Dedrick, 1984). Therefore, a pharmacokinetic model cannot yet be applied to human exposures.

3.1.5 Summary of Pharmacokinetics of PCBs

Although detailed studies of the absorption of PCBs are generally lacking, it appears PCBs may be significantly absorbed following ingestion, dermal exposure, or inhalation exposure. The highly lipophilic nature of PCBs, while making them easy to absorb, makes them difficult to eliminate. Because of their poor aqueous

solubility, PCBs are transported in blood primarily in association with chylomicrons, cellular elements, and lipoproteins. They distribute initially to tissues with high perfusion rates such as the liver and skeletal muscle, but later are redistributed to tissues with a high lipid content such as adipose tissue and skin. Attempts have been made to create pharmacokinetic models for PCB disposition based on physiological concepts. While these models have produced accurate descriptions in laboratory animals, the unavailability of good estimates for tissue metabolic clearances in man limits the utility of this approach in human exposures.

For the most part, PCBs must be metabolized to be eliminated, and it is primarily the unmetabolized PCBs which accumulate in lipoid tissues. Studies indicate mammalian cytochrome P-450 isozymes hydroxylate biphenyl in the *ortho* and *para* positions via arene oxide intermediates. Hydroxylation in the *meta* position appears to occur via a direct insertion reaction. Although mammals are capable of hydroxylating chlorinated biphenyls in any position of the ring, those isozymes responsible for *para* hydroxylation (*meta-para* arene oxide formation) tend to predominate in the liver. As chlorination substitution hinders hydroxylation of the carbon atom, the rate of PCB metabolism is dependent upon the number of substituent chlorines and their positions. This and the different isozyme patterns present in non-induced and induced animals means that differences in the elimination or bioaccumulation of the various PCB isomers may be expected. Because cytochrome P-450 isozyme patterns also vary among species, the rate and position of PCB metabolism may likewise vary substantially among species. While not discussed in this report, microbial metabolism of PCBs appears to differ substantially from mammalian systems. In microbial systems some portion of the PCBs present may be completely mineralized. Under anaerobic conditions dechlorination reactions absent in mammalian organisms predominate; with aerobic conditions hydroxylation reactions do occur but the *ortho* and *meta* position appear to be the predominant pathways.

3.2 TOXICOLOGY OF PCBS IN ANIMALS - ACUTE, SUBCHRONIC, AND CHRONIC

3.2.1 Overview of Acute Toxicity

The toxicity of PCBs has been of interest for a number of years, and several reviews of the toxic effects of PCBs in animals have been published (e.g. Fishbein, 1973; Kimbrough, 1974; Peakall, 1975; NIOSH, 1977; IARC, 1978; Kimbrough et al., 1978; USEPA, 1980; Kimbrough, 1980; Drill et al., 1982; CSWRCB, 1983; USEPA, 1985; 1987). Rather than echoing these summaries, this review will present an independent evaluation of the most relevant aspects of published information concerning the acute and subchronic toxicities of PCBs in animals.

In animals, PCBs exhibit a low order of toxicity following acute exposures. They are acutely lethal only at relatively high doses. The acute LD_{50} (the dose of a compound resulting in the death of 50% of the animals treated) for PCBs in rats and mice ranges from about 2,000 mg/kg body weight to 10,000 mg/kg body weight. According to the relative ranking system used by the American Industrial Hygiene Association, this LD_{50}-range would be classified as "slightly toxic" to "practically nontoxic" (Table 3.2.1) (Hodge and Sterner, 1949). However, as shown in Table 3.2.2, the acute lethal dosages of various PCB mixtures, such as Monsanto's Aroclors, is dependent upon the species and commercial mixture being tested. In general, the toxicological and pathological findings in rats at acutely lethal doses of PCBs include anorexia, central nervous system (CNS) depression, hepatotoxicity, nephrotoxicity, coma, and death. Hepatotoxicity is also a clinical feature in mice, rabbits, and guinea pigs when acutely lethal doses of PCBs are given (CSWRCB, 1983).

As can be seen in Table 3.2.2, the acute oral lethality of PCBs in the rat tends to decrease with an increase in chlorination. This effect has been explained by the lower oral absorption and metabolism of the more highly chlorinated PCB congeners (CSWRCB, 1983). A similar trend, however, was not observed upon dermal application of PCBs to rabbits (Table 3.2.2). Other reported trends concerning the acute toxicity of PCBs include the increased sensitivity of young animals when compared to adult animals and the greater sensitivity of the female

Table 3.2.1

A Relative Ranking System That Can Be Used to Categorize the Acute Toxicity of A Chemical

Toxicity Rating or Class	Animal LD_{50} (mg/kg)	Probable Oral Lethal Dose for Human: Approximate Lethal Dose for Average Adult	Probable Oral Lethal Dose for Human: Approximate LC_{50} (ppm) for 4-hr Inhalation in Animal
Practically nontoxic	>15,000	More than 1 quart	>100,000
Slightly toxic	5,000-15,000	Between pint and quart	10,000-100,000
Moderately toxic	500-5,000	Between ounce and pint	1,000-10,000
Very toxic	50-500	Between teaspoonful and ounce	100-1,000
Extremely toxic	5-50	Between seven drops and teaspoonful	10-100
Super toxic	<5	Less than seven drops	<10

Source: Hodge and Sterner, (1949)

Table 3.2.2

Median Lethal Doses for Various Commercial PCB Mixtures

Species	Route	Commercial PCB Mixture	LD50 (mg/kg)
Mouse	Oral	Aroclor 1254	2,000
Rat	Oral	Aroclor 1221	4,000
		Aroclor 1232	4,500
		Aroclor 1242	8,700
		Aroclor 1248	11,000
		Aroclor 1254	4,000-10,000
		Aroclor 1260	10,000
		Aroclor 1262	11,300
		Aroclor 1268	10,900
Rabbit	Dermal	Aroclor 1221	3,200
		Aroclor 1232	2,000
		Aroclor 1242	1,300
		Aroclor 1248	1,300
		Aroclor 1260	1,300-2,000
		Aroclor 1262	1,300-2,000
		Aroclor 1268	2,500

Source: James and Harbison, (1982)

when compared to the male (Kimbrough et al., 1978).

As alluded to above, there are considerable species-, strain-, sex-, and time-dependent differences in the dose that produces acute lethality. Miller (1944) found species sensitivity to Aroclor 1242 in rodents to increase in the following order: rat < rabbit < guinea pig. The acutely lethal dose in animals has been observed to decrease as the duration of the observation period increases. As seen in Table 3.2.2, the acute LD_{50} (24 hours) in rats varies between 4,000-19,000 mg/kg body weight. Bruckner et al. (1973) reported that even after 14 days, the LD_{50} was still as high as 4,250 mg/kg body weight. However, when the observation period was extended to 43 days, the LD_{75} was between 500-2,000 mg/kg body weight (Tucker and Crabtree, 1970). Similarly, Kimbrough et al. (1972) demonstrated that lethality ranged between 10 to 80% when 500-1,000 mg/kg dosages of Aroclors 1254 and 1260 were given for up to eight months. When the exposure interval was limited to six weeks, as in the study of Allen and Abrahamson (1973), neither Aroclor 1248, 1254, nor 1262 produced overt toxicity.

The primary target in animals following systemic absorption of PCBs is the liver. There, depending upon the dosage given, PCBs cause: (1) proliferation of the endoplasmic reticulum leading to an increase in the liver's microsomal drug metabolizing capacity, (2) a deposition of fat in the liver cells, and (3) cell death or necrosis. There are other organs, however, where significant toxicity also occurs. Topical or dietary exposure to PCBs may produce lesions which may include erythema, keratosis, and chloracne. In monkeys, continual oral ingestion of PCBs produces a hypertrophy and hyperplasia of the gastric mucosa with the presence of mucosal cysts and edema that is perhaps best described as a dysplastic growth pattern (Allen and Norback, 1973; Becker et al., 1979; Drill et al., 1982). Both the thymus and the immune system are also affected following systemic absorption of PCBs. Polychlorinated biphenyls have also been demonstrated to affect reproductive performance in a number of animal species and these studies will be addressed in detail in Section 3.4. The potential for teratogenic effects is also addressed in other sections.

3.2.2 Dermatotoxicity

Polychlorinated biphenyls cause dermatotoxicity following both systemic and topical application. Exposure to PCBs induces dermal changes related to hyperplasia and hyperkeratosis in a number of species including the mouse, rat, rabbit, guinea pig, and monkey. In some species, such as the monkey, dermatological effects resembling the acne-like pustules seen in humans after excessive exposure to PCBs can be produced.

Miller (1944) demonstrated that following administration of a PCB mixture (approximate chlorine content of 42%) to guinea pigs, rabbits, and rats via subcutaneous injection, feedings, and applications to the skin, there was a dilation of the capillaries of the derma and occasional thickening of the epidermis. The congestion was sometimes accompanied by edema of the derma adjacent to small blood vessels, and in one instance, a moderate infiltration of lymphocytes occurred (Miller, 1944). Miller (1944) also observed that in one experiment in which guinea pigs were painted with 3.5 to 17 mg of the PCB mixture, the primary dermal change was a thinning of the skin with destruction of the superficial, cornified layers of the epithelium. In those animals with epithelial destruction, the superficial cells contained fine, granular material and in some cases, eosinophilic inclusions. Miller (1944) noted that the reaction was not of an acute inflammation, and the amount of involvement of the skin appeared to have no relationship with the dose or length of exposure.

In contrast to the above finding of Miller (1944) that PCBs cause thinning of the skin, Vos and Beems (1971) have reported that when PCBs are applied directly to the skin of rabbits they produce a hyperplasia of both the epithelial and follicular cells causing a hyperkeratosis. With repeated applications of Aroclor 1260, these pathological changes lead to a thickening and darkening of the skin. Similar dermal lesions have been shown to occur in rats and guinea pigs after dermal application of PCBs (Drill et al., 1982).

Bell (1983) examined cutaneous tissues in mice fed Aroclor 1254. Sebaceous and hyperkeratotic changes were observed, as with other studies. Additionally, electron microscopy revealed changes in the cutaneous microvasculature. Capillaries and venules were primarily affected. Abnormalities were found in the

luminal borders of the endothelial cells, number and size of pinocytotic vesicles were increased, and basal laminae changes characterized by replicated strands or thickened amorphous layers were noted.

Puhvel et al. (1982) evaluated the dermatological effects of various chemical chloracnegens, including Aroclor 1254, Phenclor 54, and 3,4,3',4'-tetrachlorobiphenyl in two strains of hairless mice (HRS/J and Skh:HR-1). Dosages of 1 and 3 mg of Aroclor 1254, four times a week for six weeks, induced no observable changes, either grossly or histologically. On the other hand, Phenoclor 54 (0.2 mg, five times a week for 10 weeks), caused hyperkeratosis of the stratum corneum and epidermal hyperplasia, disappearance of sebaceous glands, and the presence of numerous intradermal keratinous cysts although the skin of the treated animals appeared grossly normal. Similar effects were seen in HRS/J mice treated with 0.2 mg of 3,4,3',4'-tetrachlorobiphenyl five times a week for 10 weeks.

A different pattern of dermatological effects was seen in Skh:HR-1 mice treated with 3,4,3',4'-tetrachlorobiphenyl (0.2 mg five time a week for 10 weeks). In this strain of mice, this PCB-isomer induced epidermal hyperkeratosis and hyperplasia, massive hyperkeratosis of the sebaceous follicles, and hyperkeratinization of intradermal pilar cysts which in many instances rupture, inducing the development of pimple-like areas of inflammations. Hyperkeratinization of the sebaceous follicles is considered the pathognomic lesion in human chloracne. Interestingly, these animals also underwent a gross weight gain. Weight gain is considered atypical for exposures to halogenated aromatic hydrocarbons, which usually results in weight loss and general wasting (Puhvel et al., 1982).

PCBs have also been shown to cause dermatological effects in nonhuman primates. In monkeys, cutaneous changes can be induced following oral administration of PCBs (in the diet), and these cutaneous changes are early indicators of PCB-induced intoxication in this species (Allen et al., 1974a&b; Allen, 1975). Allen et al. (1974a) fed adult female rhesus monkeys (*Macaca mulatta*) diets containing 25 ppm Aroclor 1248 for two months. One animal consumed a total PCB dose of 450 mg while the others consumed 260 mg. Within six weeks, all animals exhibited dermatological signs of toxicity including alopecia, edema of the

lips and eyelids, and moderate to severe pruritis. These effects were noticeable by the end of the first month in those animals consuming the most PCBs. By the third month, extensive alopecia was apparent, and small pustules involving hair follicles were particularly obvious around the mouth, cheeks, and neck. These acne-form eruptions are thought to arise from a squamous metaplastic change in the epithelium lining the sebaceous glands, changing the secretions of these glands from oily to keratinaceous (McConnell et al., 1979; Drill et al., 1982).

Similar findings were reported by Allen et al. (1973) in their study of subchronic toxicity of PCBs and polychlorinated terphenyls in rhesus monkeys. Animals fed 300 ppm Aroclor 1248 for 90 days developed swollen facial features, severe alopecia, and acneform skin lesions with subcutaneous edema. A slight (7%) drop in hematocrit was accompanied by gradual decreases in erythrocyte, lymphocyte, and neutrophil counts, but no liver or kidney toxicity was indicated by SGPT, SGOT, serum bilirubin, and BUN measurements. Fundic and pyloric regions of the stomach showed erosion and some necrosis, and liver hypertrophy was accompanied by increased smooth endoplasmic reticulum and modified endoplasmic reticulum surrounding lipid droplets. Allen et al. (1973) concluded that changes that occur in man and subhuman primates are quite similar, but that further study was needed.

In another experiment, Allen (1975) fed adult *Macaca mulatta* monkeys diets containing 100 and 300 ppm of Aroclor 1248 for periods ranging from 2 to 3 months. Although the total PCB intake varied between the two groups (0.8 to 1 g for the 100 ppm group and 3.6 to 5.4 g for the 300 ppm group), the signs and lesions that developed in these two groups of animals became apparent at the same time and were similar to those described above. Within three weeks to one month following the start of the experimental diet, the animals in both groups had lost a majority of their hair from the face, head, and neck, and their mouth and eyelids were edematous. Additionally, there was a loss of eyelashes, excessive lacrimation, and conjunctival congestion. Small comedones appeared around the mouth and on the cheeks and neck. Microscopically, there was development of large intrafollicular keratin cysts and epithelial hyperplasia of the hair follicles, particularly of the face with the eyelids being most severely affected. Additionally, tissues surrounding the affected hair follicles were edematous and contained acute inflammatory cells.

Similar results were observed when adult female rhesus monkeys were fed diets containing lower levels (2.5, 5, and 25 ppm) of Aroclor 1248 (Allen, 1975). The total intake of PCBs during the experiment ranged from 250 to 400 mg. Within 1 to 2 months, these animals developed periorbital edema, alopecia, erythema, and acne-form lesions that involved the face and neck. Interestingly, Allen (1975) noted that although the males consumed more PCBs than females (383 mg vs 364 mg), they exhibited only moderate periorbital edema and erythema. Allen (1975) concluded that the signs and lesions that appear in monkeys are similar to those that occur in man exposed to similar levels of PCBs in that acne, subcutaneous edema (particularly about the face), and conjunctivitis along with excessive secretion of the meibomian glands were consistently present in PCB-exposed humans and lower primates.

McConnell et al. (1979) and Altman et al. (1979) found that rhesus monkeys exposed to PCB-contaminated concrete in the slab floors of the housing facility exhibited alopecia, facial edema (especially of the lower lip and eyelids), and prominent, thickened and often tortuous meibomian glands that exuded a thick pasty material when compressed. Additionally, they observed onychogryphosis (a deformed overgrowth of the nails) that was particularly apparent in the toenails in those animals exhibiting severe alopecia. Microscopically, the nail bed was hyperkeratotic, but there was a relative lack of inflammation. Gingivitis and fibrous thickening of the mandible were also noted. A hyperkeratinization resulting in elongated, thickened, and deformed toenails has also been observed in ferrets fed Aroclor 1242 (Bleavins et al., 1982).

Hori et al. (1982) studied the effects of Kanechlor 400 (approximately 48% chlorine) with and without contaminating polychlorodibenzofurans (PCDF; 400 ppm) in female cynomolgus monkeys (*Macaca fascicularis*). Monkeys fed Kanechlor 400 contaminated with PCDFs (5-10 mg/monkey/day) exhibited considerable morbidity. In general, dermatologic symptoms in these animals included hair loss, the appearance of acne, pigmentation, periorbital edema, and hyperkeratosis. However, monkeys fed Kanechlor 400 which was PCDF-free (5 mg/monkey/day) exhibited no or only mild dermatologic signs. Thus, the dermatotoxicity of PCB mixtures may in large part be due to the presence of contaminants.

Tryphonas and colleagues have published a series of reports on the effects of Aroclor 1248 and Aroclor 1254 on rhesus and cynomolgus monkeys (Tryphonas et al., 1984; 1986a&b). Dermal or related lesions included loss of eyelashes, dilation of meibomian gland ducts, facial edema, and fingernail loss. Comparison of the cynomolgus and rhesus monkeys with respect to dermal and other symptoms of PCB toxicity indicated that the rhesus monkey may be more susceptible.

The dermatological effects of PCB mixtures in monkeys (and presumably other species) may be due to particular isomers present in the mixture rather the entire mixture itself. McNulty et al. (1980) compared the dermatological effects of diets containing 0.3 to 3.0 ppm of 3,4,3',4'-tetrachlorobiphenyl, 2,5,2',5'-tetrachlorobiphenyl, or Aroclor 1242 in adult rhesus monkeys. Only 3,4,3',4'-tetrachlorobiphenyl produced toxicities (alopecia, swelling of the lips and eyelids, and changes in the nailbeds) at the doses used. They concluded that PCB isomers unsubstituted in the *ortho*-position are more toxic than those PCB isomers that are substituted in the *ortho*-position, such as 2,5,2',5'-tetrachlorobiphenyl. This may be due to differences in receptor binding and enzyme inducing capabilities between the two PCB isomers tested (McNulty et al., 1980). A similar effect has been found in mice fed diets of various hexachlorobiphenyl isomers (Biocca et al., 1981).

3.2.3 Effects on the Liver

The effects of PCBs on the liver have been studied in a variety of laboratory animal species. In some reports, histopathologic changes have been noted, and numerous studies have found a hypertrophy of the liver which may be accompanied by an increase in the activities of some enzymes of biotransformation. Because hepatic enzyme induction *per se* may not be considered to be a toxic event, it is discussed separately from histopathologic changes associated with PCBs. Studies relating PCB exposure to the appearance of liver tumors or other neoplastic changes are discussed in Section 3.7, Carcinogenicity.

3.2.3.1 Pathology

Liver hypertrophy in response to PCB administration has been reported in each of the species of laboratory animal examined (mouse, rat, rabbit, mink, ferret, guinea pig, Japanese quail, and monkey) (Keplinger et al., 1972; Litterst et al., 1972; Allen and Abrahamson, 1973; Vos, 1972; Bruckner et al., 1973; Koller and Zinkl, 1973; Allen et al., 1973; Wolff and Hesse, 1977; Shull et al., 1982; Ignesti et al., 1986; Gillette et al., 1987a). However, its occurrence appears to depend upon the extent of chlorination, the dose, the duration of exposure, and sometimes the sex of the animal. For example, rats exposed to 100 ppm of Aroclor 1248 for 18 months experienced liver hypertrophy while the same exposure to Aroclor 1242 had no effect on liver weight (Keplinger et al., 1972). In mice, exposure to 375 or 37.5 ppm, but not 3.75 ppm, of Aroclor 1254 for 6 months resulted in increased liver weight; for Aroclor 1242, only the 375 ppm dose caused liver enlargement; Aroclor 1221 had no effect on liver weight at any of these exposure levels (Koller, 1977). Similarly, rabbits dosed once per week for 14 weeks with 300 mg of Aroclor 1254 had hypertrophied livers, while rabbits given the same dose of Aroclor 1221 for the same interval had no change in liver weight (Koller and Zinkl, 1973). Hansell et al. (1977) administered equivalent doses of four different PCB congeners to Wistar rats, one di-, two tetra-, and one hexachlorbiphenyl. Only the hexachlorobiphenyl (2,4,5,2',4',5'- hexachlorobiphenyl) produced an increase in liver weight relative to body weight.

The influence of duration of exposure on liver hypertrophy is illustrated by the study of Bruckner et al. (1973) who administered Aroclor 1242 to rats in doses of 5 and 25 ppm for two to six months. Liver hypertrophy was not evident after two months of exposure, but liver weights were significantly greater than control for both doses at four and six months. Long exposures are not always required to elicit liver hypertrophy, for Carter (1983) observed detectable increases in liver weight after only four days in Fischer rats fed Aroclor 1254 at 20 ppm in the diet. Not surprisingly, the dose of PCB administered is important in determining the extent of hypertrophy, as demonstrated by the study of Baumann et al. (1983). Rats were fed Clophen A50 for six weeks at dosages of 0, 2, 10, 50, 150, and 250 mg/kg, and a dose-dependent increase in relative liver weights was observed (Table 3.2.3).

Table 3.2.3

Effect of Clophen A50 on Liver Weight of Rats After Six Weeks of Treatment.

Dose mg/kg	Relative Liver Weight (g/100 g body weight)		
	Controls	Treated	% of Controls
2	3.27	3.35	102
10	3.42	4.12	120
50	3.50	5.07	145
150	3.14	6.58	210
250	3.50	7.36	210

Adapted from Baumann et al. (1983)

The data of Kimbrough et al. (1972) shows that sex may also be an important factor in liver hypertrophy. They observed that Aroclor 1260, when given to rats at doses of 500 or 1000 ppm in the diet for 8 months, significantly increased the liver weights of both sexes. At doses of 20 or 100 ppm this change was only noted in male animals. On the other hand, Aroclor 1254 increased liver weight in both sexes of rats at doses of 20, 100 and 500 ppm.

Histopathological changes in the liver associated with PCBs also appear to be dependent on the commercial mixture or isomer used, dose, and length of exposure. Enlarged hepatocytes and proliferation of the smooth endoplasmic reticulum are common, and are associated with hypertrophy of the liver (Allen and Abrahamson, 1973; Hansell et al., 1977; Gillette et al., 1987a; Hinton et al., 1978). The increase in smooth endoplasmic reticulum appears to be a consequence of increases in both microsomal protein and phospholipids. Hinton et al. (1978) has suggested that the increase in microsomal proteins may arise from both an increase in synthesis and a decrease in catabolism. While no obvious changes in

phospholipid synthesis were noted following PCB exposure, the half-life of labelled phospholipid was increased in PCB-treated animals.

Lipid vacuolization has been noted in a number of studies. For example, Allen and Abrahamson (1973) fed rats 1000 ppm Aroclor 1248, 1254, and 1262. Histopathologic examination of all of the Aroclor-treated animals showed small fat droplets. Similar lipid accumulation was seen by these investigators in a later study using lower dosage exposure to commercial PCB mixtures (Allen et al., 1976). Bruckner et al. (1973) used much smaller doses of Aroclor 1242 in rats, and reported very small lipid-containing (Sudan IV staining) vacuoles after 2, 4, or 6 months exposure to 5 or 25 ppm. Dose- and isomer dependence of this effect is shown by the data of Biocca et al. (1981). In their study, mice fed 3,4,5,3',4',5'-hexachlorobiphenyl for 28 days showed fatty metamorphosis of the liver at 10 and 30 ppm doses, but not at 1 or 3 ppm. Mice fed 300 ppm of the 2,4,6,2',4',6'-hexachlorobiphenyl isomer also had fatty metamorphosis, but the same dose of 2,4,5,2',4',5'-hexachlorobiphenyl or 2,3,6,2',3',6'-hexachlorobiphenyl did not produce the fatty liver changes.

The appearance of fatty liver has been paralleled by measurements indicating an increase in total lipid content of the liver of PCB-treated animals (Kluwe et al., 1982). More detailed studies of lipid content reveal a modest increase in phospholipids, a more substantial increase in triglycerides, and a decrease in cholesterol (Litterst et al., 1972; Allen and Abrahamson, 1973; Hinton et al., 1978; Allen and Abrahamson, 1979). The increase in triglyceride appears to be the result of impaired utilization by the liver rather than increased synthesis (Hinton et al., 1978).

In some species the fatty liver effect of PCBs may be an indirect effect. Mink were administered 3,4,3',4'-tetrachlorobiphenyl, 50 mg/kg ip for three days, and sacrificed seven days after the initial injection (Gillette et al., 1987b). There were two control groups; both received vehicle and one was pair-fed with the PCB treatment group, since food consumption of the PCB-treated mink was decreased. Livers from both the 3,4,3',4'-tetrachlorobiphenyl group and the pair-fed controls exhibited moderate to severe fatty liver change ranging from the periportal region

to the entire lobule. The untreated controls (vehicle only, no food restriction) were normal, indicating that the peanut oil vehicle used to administer the PCB could not have caused the fatty changes. The identical histopathologic response in the pair-fed controls suggests that the fatty changes in mink were the result of altered nutritional status rather than a direct effect of the PCB.

A few animal studies have described hepatic necrosis as a consequence of exposure to PCBs. Allen and Abrahamson (1973) found focal necrosis in livers of rats fed 1000 ppm of three different Aroclors (1248, 1254, 1262) for six weeks. Baumann et al. (1983) fed rats Clophen A50 for six weeks in doses of 0, 2, 10, 50, 150, and 250 mg/kg. Disseminated necroses were observed in the livers from animals in all treatment groups. Both single cell and focal necroses were found, with the focal necroses preferentially located in the centrilobular region. Infiltration of polymorphonuclear granulocytes into the focal necroses was noted. Andersen et al. (1985) also reported hepatic necrosis among mice fed Clophen A60 in the diet in concentrations ranging from 10 - 200 ppm for approximately 20 weeks. Unfortunately, interpretation of their observations is confounded by the fact that mice in each PCB treatment group also received a radiolabelled dose of cadmium.

As with other hepatic effects of PCBs, the incidence and severity of hepatic necrosis seem to be dose- and isomer- dependent. Jonsson and others fed female rats Aroclor 1242 at 75 or 150 ppm for 36 weeks and noted focal necrosis in the PCB-treated animals (Jonsson et al., 1981). Necrosis was more pronounced in animals receiving the higher dietary PCB level. In another study, mice fed 300 ppm of 3,4,5,3',4',5'- and 2,4,6,2',4',6'-hexachlorobiphenyl showed histopathologic signs of necrosis while mice fed the same dose of the 2,4,5,2',4',5'- and 2,3,6,2',3',6'- isomers had no necrotic lesions (Biocca et al., 1981). Hansell et al. (1977) found that while a single intraperitoneal injection of 2,4,5,2',4',5'-hexachlorobiphenyl (0.2 mmol/kg) produced extensive centrilobular necrosis in rats after 35 days, similar doses of 4,4'-dichlorobiphenyl, 2,4,2',4'-tetrachlorobiphenyl, and 2,5,2',5'-tetrachlorobiphenyl did not produce necrosis. The rabbit seems to be more susceptible to necrosis than the rat (Drill et al., 1982), and Vos and Beems (1971) found hepatic necrosis after dermal exposure

to 120 mg of Aroclor 1260, 5 times a week for 4 weeks. A dependence upon the extent of chlorination for necrosis was seen in another study of rabbits using Aroclors 1254, 1242, and 1221 (300 mg once per week for 14 weeks) (Koller and Zinkl, 1973). Rabbits treated with Aroclor 1254 developed hepatic necrosis, Aroclor 1242 produced less necrosis, and no necrosis appeared in rabbits given the same regimen of Aroclor 1221. Similar effects were seen in mice treated with Aroclor 1254, 1242, or 1221 (Koller, 1977).

Some investigators have reported the appearance of a brown pigment in the livers of animals treated with PCBs. Jonsson et al. (1981) observed a marked accumulation of an iron-containing pigment in hepatocytes and Kupffer cells of PCB-treated rats, and Kimbrough and coworkers (Kimbrough et al., 1972;1973) also found brown pigment in macrophages and Kupffer cells. Yagi et al. (1985) identified the dark-brown pigmentation histochemically as a ceroid produced by peroxidized lipid or lipoprotein. Increased lipid peroxidation has been observed and examined in association with PCB exposure by a number of investigators (e.g. Saito et al., 1983; Kamohara et al., 1984).

Kimbrough and coworkers have reported other histopathologic abnormalities among Sherman rats chronically exposed to PCB mixtures. In 1972, they reported the results of a study examining the effects of Aroclors 1254 and 1260 fed to both male and female Sherman rats at various dietary levels for eight months (Kimbrough et al., 1972). The overall toxicity of the exposures is indicated by their mortality data. The females were more sensitive to Aroclor 1260: 1/10 died at 100 ppm, 2/10 died at 500 ppm, and 8/10 died at 1,000 ppm. No male animals died at any dose level. Aroclor 1254 was approximately equally toxic to both male and female rats as 2/10 male and 1/10 female animals died at 500 ppm while 5/10 male and 8/10 female animals died at 1,000 ppm; no animals of either sex died at dosage levels below 500 ppm. The histopathologic findings included hepatocellular hypertrophy and lipid accumulation associated with a foamy cytoplasm, observations consistent with a number of other reports. They also noted an accumulation of a pigment containing hemosiderin in the Kupfer cells, and adenofibrosis was present at the higher dietary levels. Both Aroclors produced similar effects, but Aroclor 1254 appeared to be more hepatotoxic in view of the greater incidence of

adenofibrosis found at lower dietary exposures and the fact that, in general, the hepatic changes were more pronounced in the Aroclor 1254-exposed animals.

In subsequent publications (Kimbrough et al., 1973; Rao et al., 1986) Kimbrough and coworkers reported that 15 of 35 Sherman rats fed Aroclor 1254 at a dietary concentration of 500 ppm for six months had pancreatic-type tissue in their livers. The cytoplasm of this tissue contained esterases and tryptophan, as detected by histochemical stains. Two enzymes normally secreted by pancreatic acinar cells, amylase and trypsinogen, were found in this tissue using a protein A-gold immunocytochemical method. This tissue was determined to be morphologically and functionally identical with normal pancreatic acinar tissue, and was usually associated with areas of adenofibrosis. The precursor cells for this tissue are unknown, as is the mechanism for this transdifferentiation.

Kimbrough et al. (1973) have also tried to determine whether or not the hepatic changes observed in their studies would regress with time. Fifty Sherman rats were fed diets containing 500 ppm of Aroclor 1254 for six months. The animals were then returned to a normal diet, and groups of animals were sacrificed at various time intervals over the next 10 months. Except for the disappearance of epithelial cells from the center of the lesions, no regressions of the adenofibrosis were noted. Liver weight returned to normal during the 10 month period without exposure, but the lipid accumulation and hypertrophy remained. While these results might suggest that the hepatic effects are not reversible, the body burdens of PCBs still remained excessive for the entire 10 months without PCB exposure. Analysis of the adipose tissues taken from the last group sacrificed revealed that the PCB levels ranged from 924-1688 ppm with a mean of 1192 ppm. An analysis of the livers found PCB levels that ranged from 17-26 ppm with a mean of 22.7 ppm. These tissue levels indicate a continuing exposure of the liver to PCBs throughout the study period. Conclusions regarding the ability of the liver to recover in the absence of continued exposure therefore cannot be drawn from this study.

Burse et al. (1974) performed a similar experiment by exposing rats for four to six months to Aroclors 1016 and 1242. These investigators also noted a

persistence of the PCBs in adipose tissue after discontinuation of the diet; Aroclor 1016 was detectable for five months and 1242 for six months after exposure. While the hypertrophy remained after cessation of Aroclor exposure, the frequency of vacuolization or cytoplasmic inclusions decreased, suggesting that these hepatic effects are in fact reversible with time.

Koller (1977) found that liver lesions in mice induced by six months exposure to Aroclor 1242 had regressed by 3 months post-exposure. Aroclor 1254-induced hepatic lesions were, however, still evident after the three month recovery period. Differences in residual levels of Aroclor 1254 and Aroclor 1242 may explain the observed difference in regression.

3.2.3.2 Induction of Metabolism by PCBs

Biotransformation is a process whereby the structures of endogenous and exogenous compounds are altered through enzymatic activity. When the substrate for a biotransformation reaction is a drug or other exogenous compound, this phenomenon is sometimes termed drug metabolism or xenobiotic metabolism (xenobiotic meaning foreign compound). It is thought that the objectives of these biochemical reactions are to make the chemical less biologically active and easier to eliminate. The change in biological activity is a consequence of an alteration in chemical structure and loss of the structural attributes responsible for the chemical's biological effects. Enhanced elimination is brought about, in general, by changes which increase the water solubility of the compound.

Biotransformation, or xenobiotic metabolism, is a naturally occurring and necessary process that helps the body maintain homeostasis and eliminate those foreign substances to which we are continually exposed. It occurs primarily in the liver, lungs, and kidneys; the liver, because of its greater capacity for metabolism, is generally the most important of these. Often, slowly metabolized chemicals can cause an increase in their own metabolism. This change is the result of an increase in the activity of enzymes responsible for biotransforming xenobiotics. The increase in enzyme activity is usually called enzyme induction, or more appropriately the induction of metabolism or biotransformation. The induction

process is usually not highly substrate specific, and the biotransformation of other compounds metabolized by the induced enzymes may be increased as well.

Enzyme induction is a common property of organochlorine compounds; Street (1969) were the first to report hepatic enzyme induction after exposure of animals to PCBs. Since this report, the enzyme-inducing properties of the PCBs have been studied extensively (e.g, Villeneuve et al., 1971b; Litterst and Van Loon, 1972; Litterst et al., 1972; Villeneuve et al., 1972; Alvares et al., 1973; Chen and DuBois, 1973; Litterst and Van Loon, 1974; Goldstein et al., 1974; Johnstone et al., 1974; Turner and Green, 1974; Vainio, 1974; Alvares and Kappas, 1975; Bickers et al., 1975; Ecobichon, 1975; Ecobichon and Comeau, 1975; Bickers, 1976; Burse et al., 1976; Bickers et al., 1977; Bruckner et al., 1977; Goldstein et al., 1977; Hansell et al., 1977; Marniemi et al., 1977; Wolff and Hess, 1977; Birnbaum and Baird, 1978; Hinton et al., 1978; Riviere et al., 1978; Allen and Abrahamson, 1979; Alvares and Kappas, 1979; Narbonne, 1979; Matthews and Kato, 1979; Carlson, 1980; Narbonne, 1980; USEPA, 1980; McKinney and Singh, 1981; Ueng and Alvares, 1981; Iverson et al., 1982; Shull et al., 1982; Kamataki et al., 1983; Parkinson et al., 1983; Neskovic et al., 1984; Safe, 1984).

Early studies, such as the one by Fujita et al. (1971), found differences in the extent of induction produced by different PCB congeners. The rates of *in vitro* microsomal metabolism of marker compounds subject to metabolism by the enzyme cytochrome P-450 were used to measure induction, as well as the duration of action of a barbiturate (barbiturate sleep time). They concluded that both the extent and position of chlorination are important in determining the magnitude of induction by PCBs of these cytochrome P-450 mediated activities.

Johnstone et al. (1974) were among the first to report that the induction of hepatic enzyme activities by PCBs is not confined to activities related to cytochrome P-450. Young male Wistar rats were administered mono-, di-, tetra-, hexa-, and octachloro- congeners of PCB intraperitoneally for 3 days. Rates of aniline hydroxylase, O-demethylase, N-demethylase, nitroreductase, carboxylesterase activities, and the rate of BSP conjugation with glutathione were measured in appropriate hepatic subcellular fractions derived from these animals. Hepatic

induction *in vivo* was also assessed by barbiturate sleep time. PCB congeners produced changes indicating induction in each of these indices, although there were important differences among congeners. For example, O-demethylase was induced by hexachloro- and octachlorobiphenyl congeners, and by tetrachlorobiphenyl substituted in the 2 and 4 positions (2,2',4,4'-tetrachlorobiphenyl) but not the 2 and 5 positions (2,2',5,5'-tetrachlorobiphenyl). Dichlorobiphenyl also induced O-demethylase activity, but only when substituted in the 4 position (4,4'-dichlorobiphenyl) and not the 2 position (2,2'-dichlorobiphenyl). The types of enzyme activities induced by congeners were also variable. Octachlorobiphenyl, for example, produced induction indicated by each of the parameters measured. 4-Monochlorobiphenyl, on the other hand, induced only BSP conjugation. It was concluded from these data that the higher chlorinated congeners are more effective inducers, and that the position of chlorination of some congeners was very important for enzyme induction.

Subsequent investigations have further defined the diversity of enzymatic activities that may be induced following exposure to PCBs. Metabolism not only of xenobiotics but also endogenous compounds may be enhanced (e.g. Yoshimura et al., 1985). Examples of enzyme activities induced by PCBs appear in Table 3.2.4.

The importance of the position of chlorination in induction was shown in a subsequent study (Ecobichon and Comeau, 1975). Biphenyl, three monochlorobiphenyl congeners, four dichlorobiphenyls, five trichlorobiphenyls, five tetrachlorobiphenyls, one pentachlorobiphenyl, two hexachlorobiphenyls, and one octachlorobiphenyl were examined with respect to their ability to produce induction, as indicated by the same parameters used in the previous study (Johnstone et al., 1974) in Wistar rats. Position and degree of chlorination did not appear to be important for effects on BSP conjugation or carboxylesterase activity, as nearly all of the congeners increased the activity of these reactions. Significant differences among congeners appeared for the other enzyme activities. The authors observed that for enzyme activities closely associated with the endoplasmic reticulum, such as those related to cytochrome P-450, the 4 and 4' position chlorine substitution appears to be important. For those enzymatic activities less closely

Table 3.2.4

Examples of Enzymatic Activities Induced by PCBs

benzo[a]pyrene hydroxylases
dibenz[a,b]anthracene hydroxylases
pyrene hydroxylases
phenanthrene hydroxylases
chrysene hydroxylases
ben[a]anthracene hydroxylases
4-halobiphenyl hydroxylases
biphenyl 2- and 4- hydroxylases
acetanilide hydroxylase
warfarin hydroxylases
aflatoxin B1 hydroxylases
7-ethoxyresorufin O-deethylase
p-nitroanisole O-demethylase
substituted aromatic ether O-dealkylases
barbiturate hydroxylases
dimethylaminoantipyrine N-demethylase
ethylmorphine N-demethylase
benzphetamine N-demethylase
glucuronyl transferases
epoxide hydrolase
glutathione transferases

Source: Safe, (1984)

associated with the endoplasmic reticulum, e.g. BSP conjugation and carboxylesterase activity, the position of chlorination is much less important.

While the extent and position of chlorination of PCB congeners is important in determining the extent of cytochrome P-450 induction, they are also important in determining the type of cytochrome P-450 induction. Enzyme-inducing agents have historically been divided into two groups, those that have an enzyme induction effect similar to phenobarbital and those whose induction is similar to

that produced by polycyclic aromatic hydrocarbons, e.g. 3-methylcholanthrene (Sladek and Mannering, 1966; Conney, 1967). This distinction was made when it was recognized that these two agents induce different major forms of cytochrome P-450.

Initial studies with PCBs suggested that they represented a new type of inducing agent because they were the first compounds shown to be capable of causing enzyme induction which resembled both the phenobarbital- and 3-methylcholanthrene types (Alvares et al., 1973). These studies involved the administration of PCB mixtures. Subsequent studies of individual congeners revealed that some PCB congeners were phenobarbital-type inducers while other congeners were 3-methylcholanthrene-type inducers. This is illustrated by the report by Goldstein et al. (1977). They administered commercial PCB mixtures and a variety of different individual PCB congeners to female CD rats and determined the effect on aryl hydrocarbon hydroxylase, aminopyrine N-demethylase, and glucuronyl transferase activity, as well as cytochrome P-450 content and other parameters that related to the cytochrome P-450 enzyme. Based upon their observations, these investigators concluded that biphenyls symmetrically chlorinated in both the *meta* and *para* positions produced induction similar to 3-methylcholanthrene. Biphenyls with chlorination in both the *ortho* and *para* positions produced induction resembling phenobarbital. Congeners chlorinated in only one ring, or chlorinated in both rings but not in the *para* positions have little effect as inducers.

For both the phenobarbital-type and the 3-methylcholanthrene-type chlorobiphenyls, the degree and position of chlorination influence the extent of induction. The study of Denomme et al. (1983) illustrates this principle for congeners with induction similar to phenobarbital. A series of symmetrical and asymmetrical PCB congeners with chlorine substitution at positions 2 and 4 was tested for inducing effects in immature male Wistar rats. Microsomal enzyme activities monitored included dimethylaminoantipyrine N-demethylase, benzo[a]pyrene hydroxylase, aldrin epoxidase, and ethoxyresorufin O-deethylase. All were verified to produce phenobarbital-like inductive effects. The most effective inducing congeners contained at least two *ortho* and two *para* chlorine

substituents. Addition of a third *ortho* chlorine diminished induction activity, as determined by comparison of effects of 2,2',4,4'-tetrachlorobiphenyl with 2,2',4,4',6-pentachlorobiphenyl and 2,2',3,4,4',5-hexachlorobiphenyl with 2,2',3,4,4',5,6-heptachlorobiphenyl. *Meta* position chlorination was also important, and nearly always resulted in an increase in induction effects.

Structure-activity relationship studies have also been performed for congeners which produce 3-methylcholanthrene-like induction, and there has been a great deal of research in defining the mechanism of this induction. It appears that the biochemical processes leading to this type of induction are initiated by the combination of the PCB congener with a specific cytosolic receptor. The PCBs are not unique in combining with this receptor, and several aromatic hydrocarbons have been shown to have affinity. Combination with this receptor is thought to result in an interaction with a genomic regulatory element resulting in a stimulation of the transcription of a cytochrome P-450 gene. This particular form of cytochrome P-450 is responsible for, among other enzyme activities, aryl hydrocarbon hydroxylase (AHH). The genetic locus controlling this protein synthesis is termed the *Ah* locus (for Aromatic Hydrocarbon), and the cytosolic receptor has been called the *Ah* receptor (Nebert and Gielen, 1972; Nebert et al., 1972; Thomas et al., 1972).

Both tetrachlorodibenzofuran (TCDF) and tetrachlorodibenz-*p*-dioxin (TCDD) have a relatively high affinity for the *Ah* receptor, and both are planar molecules. It was therefore originally postulated that PCB congeners such as 3,4,5,3',4',5'-hexachlorobiphenyl and 3,4,3'4'-tetrachlorobiphenyl assume a planar conformation which permits them to bind to the receptor. McKinney and Singh (1981) examined the X-ray crystalline structure of these congeners and found that their preferred conformation was significantly non-planar with a dihedral angle of approximately 47°. Depending upon the assumptions used in the calculation, these authors estimated that only 1 to 18% of these PCB molecules should exist in the planar configuration. Assuming that only molecules in the the planar configuration can bind to the receptor, this may account for at least part of the differences in potency between PCBs, TCDF, and TCDD as observed by Nagayama et al. (1983).

The importance of the position of chlorination of PCBs in terms of effectiveness can be seen by comparison with the structure of TCDD, which has a very high affinity for the *Ah* receptor. An overlay of the planar form of a 3,4,3',4'-substituted PCB and TCDD reveals that the 4 and 4' chlorines of the PCB superimpose precisely on two of the planar chlorines of TCDD. This is consistent with a requirement of symmetrical *para-* substitution in PCBs for 3-methylcholanthrene-like induction. One of the two *meta-* substituted chlorines of 3,4,3',4'-tetrachlorobiphenyl is in proximity with the remaining planar chlorines of TCDD, whereas two of the four *meta-* substituted chlorines of 3,4,5,3'4,',5'-hexachlorobiphenyl are in proximity. The greater number of chlorines in a spatial arrangement similar to that of TCDD perhaps explains why 3,4,5,3',4',5'-hexachlorobiphenyl is more effective in binding than 3,4,3',4'-tetrachlorobiphenyl (McKinney and Singh, 1981).

McKinney and Singh (1981) further proposed that planarity is not a requirement for binding to the receptor, *per se*. They suggested that the underlying requirement is net polarizability of the molecule. Four lateral chlorines appear to be required for sufficient net polarization of the molecule to permit binding. For some molecules, such as the 3 and 4 substituted tetra- and hexachlorobiphenyls described above, planarity is required only in that it places the lateral chlorines in the position to achieve sufficient net polarization. *Ortho* substitution reduces the percentage of molecules in the planar configuration, and therefore reduces binding. Binding to the *Ah* receptor has also been suggested as an important step in producing toxic effects from PCBs. A further discussion of *Ah* receptor binding in this context appears in Section 3.3, Mechanisms of Toxicity.

Some confusion has been generated by reports that some individual PCB congeners can produce induction which resembles both 3-methylcholanthrene and phenobarbital. This was reported by Alvares and Kappas (1977) for 2,4,5,2',4',5'-hexachlorobiphenyl and by Stonard and Greig (1976) for 2,3,4,2,',3',4'-hexachlorobiphenyl. Goldstein et al. (1977) examined these congeners and found that their induction was purely of the phenobarbital type. Goldstein et al. (1978) subsequently published a study which found that a commercial

preparation of 2,4,5,2',4',5'-hexachlorobiphenyl contained a small contaminating amount of tetrachlorodibenzofuran (TCDF). TCDF produces induction resembling 3-methylcholanthrene. Although the commercial congener was >99% pure, the contaminating TCDF was sufficiently potent as an inducer to account for the 3-methylcholanthrene-like effects attributed to the PCB congener. To confirm this, Goldstein et al. added 50 ppm TCDF to purified 2,4,5,2',4',5'-hexachlorobiphenyl and administered the combination to rats. The induction response was indistinguishable from that produced by the commercially-available 2,4,5,2',4',5'-hexachlorobiphenyl (>99% pure) congener.

It is understandable that commercial mixtures of PCB congeners result in a "mixed-type" induction pattern containing elements of both phenobarbital and 3-methylcholanthrene induction. However, TCDF contamination may not account for all of the observations of a "mixed" type induction produced by an individual congener (e.g. reports by Dannan et al., 1978 and Parkinson et al., 1980a). It is possible that a single congener may have structural attributes that enable it both to bind with the *Ah* receptor and also initiate the cellular events leading to phenobarbital-like induction (McKinney and Singh, 1981). Although studies such as the one by Denomme et al. (1983) have begun to describe structure-activity relationships for PCB congeners producing phenobarbital-like induction, there has been difficulty in establishing the overall structure-activity relationships among all compounds producing phenobarbital-like induction. Further, the mechanism by which such induction occurs is not well understood. While it is tempting to speculate that there is a phenobarbital receptor analogous to the *Ah* receptor, the evidence to date neither supports nor refutes this concept (Whitlock, 1986). Until the mechanism of phenobarbital-type induction is better understood, the relationship between it and 3-methylcholanthrene induction, especially as it relates to PCBs, will be unclear.

Detailed biochemical studies of cytochrome P-450 have revealed that there are several different forms of the enzyme, or isozymes, in the liver of rats. Phenobarbital induction typically increases the levels of the related isozymes cytochromes P-450b and P-450e. 3-Methylcholanthrene preferentially induces cytochrome P-450c, and other substances induce cytochromes P-450a and P-450d.

Pretreatment of rats with PCB mixtures was found to induce all five of these isozymes of cytochrome P-450 (Ryan et al., 1977; Ryan et al., 1979; Botelho et al., 1979, Thomas et al., 1981; Ryan et al., 1982), although not equally. For example, Parkinson et al. (1983a&b) examined the time course of induction of specific cytochrome P-450 isozymes following the administration of a single large dose (500 mg/kg) of Aroclor 1254. As had been observed previously, cytochromes P-450a, P-450b + P-450e, P-450c, and P-450d were all increased by Aroclor pretreatment. [Note: Methodology used in this study could not distinguish between cytochromes P-450b and P-450e, so the levels were reported as the sum of these two isozymes.] The time courses of the increases were sometimes very different among isozymes, however. Cytochromes P-450c, P-450d, and especially P-450b + P-450e increased rather quickly and were at maximal levels by days 3-5. Cytochromes P-450b + P-450e declined quickly on days 4-9, remained relatively constant for about five days, and then began a slow decline. The initial rapid decline was substantial enough to cause an approximate 25% decline in total cytochrome P-450 during this period. Levels of cytochromes P-450d and P-450c remained relatively constant from days 5-15, and then they too began to decline. Induction of cytochrome P-450a was less extensive, though greater than that previously reported to occur after either phenobarbital or 3-methylcholanthrene induction. Levels of cytochrome P-450a reached a plateau at approximately day four and remained relatively constant until approximately day 15, when levels began to decline. Epoxide hydrolase content was increased considerably, and the time course matched that of most of the isozymes. Peak levels were reached by approximately day five, levels remained constant until approximately day 15, and then began to decline.

Parkinson et al. (1983b) wanted to determine whether the decline in cytochromes P-450b + P-450e was due to preferential early elimination of the specific PCB congener(s) responsible for the induction of these isozymes. They tested two PCB congeners that had previously been shown to produce both phenobarbital- and 3-methylcholanthrene-like induction, 2,3,4,5,3',4'-hexachlorobiphenyl and 2,3,4,5,4'-pentachlorobiphenyl. Each of these congeners produced a rapid rise in cytochromes P-450b + P-450e, followed by the same transient decline on days 4-9 as was observed with the Aroclor treatment. Hepatic concentrations of the congeners remained relatively constant during the day 4-9

period. Based upon these observations, the authors concluded that the decline was not due to preferential elimination of isozymes responsible for the induction of cytochromes P-450b + P-450e. When 2,4,5,2',4',5'-hexachlorobiphenyl (a congener which induces cytochromes P-450b + P-450e and P-450a but not P-450c or P-450d) was administered, the transient decrease was not seen.

Scholte et al. (1985) were able to distinguish between cytochromes P-450b and P-450e and determined their relative induction in Wistar rats treated phenobarbital, 4,4'-dichlorobiphenyl, and 2,4,5,2',4',5'-hexachlorobiphenyl. Phenobarbital and the hexachlorobiphenyl treatment resulted in a faster and more extensive increase in the contents of cytochromes P-450b and P-450e. Phenobarbital induced both cytochromes P-450b and P-450e, while the hexachlorobiphenyl induced primarily cytochrome P-450b.

A recent study of the effects of 3-methylcholanthrene and 3,4,5,3',4',5'-hexachlorobiphenyl on male-specific rat hepatic cytochrome P-450 2c was conducted by Yeowell et al. (1987). They demonstrated that within 7 days after PCB or 3-methylcholanthrene administration at 50 mg/kg, rat liver cytochrome P-450c activity was significantly increased while the male-specific cytochrome P-450 2c activity was significantly decreased. These investigators linked the decrease in P-450 2c activity to a decreased level of hepatic mRNA which codes for this male-specific enzyme. Yeowell et al. (1987) concluded that 3-methylcholanthrene and hexachlorobiphenyl may decrease transcription of mRNA for P-450 2c as a consequence of binding with the *Ah* receptor, or may interfere with the hormonal regulation of the mRNA for P-450 2c. However, because their studies on mRNA were exclusively *in vitro*, they could not rule out the possibility that a decrease in translatable message was not the cause of their observed decrease in P-450 2c mRNA translational efficiency.

The extent of hepatic microsomal enzyme induction following PCB exposure appears to be dose-related, and minimum effective doses in rats have been reported for two commercial mixtures. Litterst and Van Loon (1972) fed male Osborne-Mendel rats Aroclor 1254 at doses of 0.05, 0.5, or 4.7 mg/kg/day for 30 days. Significant induction, as determined by cytochrome P-450 content and

demethylase, reductase, and hydroxylase activities was observed at the 4.7 mg/kg dosage only. Turner and Green (1974) also fed Aroclor 1254 to rats in dosages ranging from 0.1 to 10 ppm for 12 weeks. Induction was noted among rats fed 10 ppm, but not 0.1 or 1.0 ppm. Bruckner et al. (1974a) administered a single dose of Aroclor 1242 intraperitoneally to male Sprague Dawley rats in dosages ranging from 1 to 100 mg/kg. No increase in hydroxylase activity, N-demethylase activity, or cytochrome P-450 content was observed following the 1 mg/kg dose. Only a modest increase in hydroxylase activity was noted at the 5 mg/kg dosage, but hydroxylase and demethylase activities were both significantly elevated at dosages of 25 mg/kg or greater.

The report by Villeneuve et al. (1971b) provides some estimate of the minimum effective dose of PCBs for hepatic enzyme induction in rabbits. These investigators administered either Aroclor 1221 or Aroclor 1254 daily to pregnant rabbits in dosages of 1.0 or 10 mg/kg for 28 days. Carboxylesterase, aniline hydroxylase, and aminopyrine N-demethylase were measured, along with paraoxon degradation. Aroclor 1221 administration did not cause enzyme induction at either dosage. Aroclor 1254 produced enzyme induction at 10, but not 1.0 mg/kg/day. The authors concluded that the minimum effective dosage of Aroclor 1254 for enzyme induction in the pregnant rabbit is between 1 and 10 mg/kg/day, and that the minimum dose for Aroclor 1221 must be higher than 10 mg/kg/day.

Signs of hepatic enzyme induction may appear relatively early following exposure to PCBs. Litterst and Van Loon (1974) found significant increases in cytochrome P-450 content, demethylation, and nitroreduction as early as 12 hours after the administration of a single oral dose of Aroclor 1254, 50 mg/kg. Peak activity occurred at approximately 24 hours after the dose and then slowly returned to normal levels.

Aroclor 1254 was found to shorten the half-life of pentobarbital, a drug metabolized primarily by liver microsomal enzymes (Chu et al., 1977). Rats were administered 0, 1, 5, or 25 ppm Aroclor 1254 in the diet for up to 140 days prior to injection with pentobarbital. After 35 days of pretreatment, only the 25 ppm group

showed a significant acceleration of pentobarbital elimination. After 70 or 140 days of Aroclor treatment, both the 5 and 25 ppm groups showed increased pentobarbital elimination. Aroclor treatment decreased the elimination rate and clearance of pentobarbital but did not alter the distribution of the drug, which indicates that the primary effect was through induced liver metabolism. This subchronic study in rats indicates that the minimum effect dose level for microsomal enzyme induction is in the range of 1-5 ppm.

PCBs appear to have enzyme inducing effects in all species examined, including mice, rats, guinea pigs, hamsters, rabbits, mink, ferrets, pigs, monkeys, owls, Japanese quail, cockerels, and fish (e.g., Shull et al., 1982; Wolff and Hesse, 1977; Ignesti et al., 1986; Miranda et al., 1987; Iverson et al., 1982; Hansen et al., 1981; Bunyan and Page, 1978; Lake et al., 1979; Rinsky and Perry, 1981; Addison et al., 1978; Guoth et al., 1984). Induction has also been demonstrated *in vitro* in cultured hepatoma cells (Sawyer and Safe, 1985). There appears to be variability among these species with respect to sensitivity and the type of induction response. For example, Wolff and Hesse (1977) examined the effects of Clophen A-50 on xenobiotic metabolism in rabbits and rats. Treatment with 50 mg/kg daily for five consecutive days resulted in substantial increases in liver weights and cytochrome P-450 concentrations in both rat and rabbit. In the rabbit, however, enzyme activities, all primarily associated with phenobarbital-type induction, were unchanged. The enzyme activities measured were p-nitroanisole O-demethylase, aminopyrine N-demethylase, aniline hydroxylase, and 4-chlorobiphenyl hydroxylase activities. All of these enzyme activities were significantly increased in the rat by the Clophen A50 treatment. Similar differences were observed with respect to effects of Clophen A50 treatment on *in vivo* hexobarbital metabolism. The rabbit is not the only species which has been shown to respond to commercial PCB mixtures with only, or nearly only, 3-methylcholanthrene-type induction. Similar observations have been made with Aroclor 1016 or 1242 exposure to mink and ferrets (Shull et al., 1982), and in a number of studies of PCB induction effects in fish (Safe, 1984).

Enzyme induction is not confined to the liver, although the liver is the tissue most frequently examined. The placenta, lungs, skin, and kidneys have also been

shown to be sites for enzyme induction in response to exposure to PCBs (Alvares and Kappas, 1975; Ueng and Alvares, 1981; Bickers et al., 1975; Vainio, 1974; Ghazarian et al., 1980; Mitchell et al., 1987; Ueng and Alvares, 1985; Wong et al., 1986 a&b;1987). Studies by Serabijt-Singh et al. (1983) have revealed an interesting contrast in the effect of PCB induction on rabbit liver versus rabbit lung. While Aroclor 1260 treatment (200 mg/kg, 144 and 96 hours prior to sacrifice) increased cytochrome P-450 form 6 (one of three isozymes of cytochrome P-450 identified in rabbit lung) in both lung and liver, cytochrome P-450 form 5 was increased in the liver only. Further, Aroclor 1260 treatment caused a modest increase in form 2 in the liver, but almost a complete loss of form 2 in the lung. It was found that the opposite response of liver and lung form 2 to Aroclor 1260 treatment was due to the difference in response of this form to stimulation by phenobarbital-like inducers. Cytochrome P-450 form 2 responds (increases content) to phenobarbital in the liver, but not in the lung. Aroclor 1260 apparently contains congeners which are repressive to form 2. This repression in the liver is antagonized by other congeners in Aroclor 1260 which stimulate this form by virtue of their phenobarbital-like inductive effects. Because the lung form 2 does not respond to phenobarbital-like inducers, there is no antagonism of the repressive effect of Aroclor 1260 in this organ and the form 2 content decreases dramatically.

Birnbaum and Baird (1978) examined the ability of PCBs (Aroclor 1254) to produce hepatic enzyme induction in senescent rats. Cytochrome P-450 content was measured, and ethylmorphine N-demethylase, benzphetamine N-demethylase, and benzo[a]pyrene hydroxylase activities were determined in young, middle aged, and old rats. Although xenobiotic metabolizing capability was generally somewhat diminished in the old rats, they responded to Aroclor treatment with induction not unlike the younger animals. An unchanged responsiveness with advanced age has also been shown for the inducing compounds phenobarbital, 3-methylcholanthrene, and pregnenolone-16 alpha-carbonitrile (Birnbaum and Baird, 1978).

It should be recognized that PCB exposure does not always result in increased metabolism; Rosin and Martin (1983b) have shown that PCBs may either enhance or diminish hepatic biotransformation reactions, depending upon the

timing of the dosing. Male CD-1 mice were administered Aroclor 1254 (500 mg/kg) by gavage at varying times prior to the administration of pentobarbital, and the duration of sleep time in response to the pentobarbital was measured. Co-administration of Aroclor 1254 with the pentobarbital produced a small increase in sleep time, but larger increases in sleep time were noted as the interval between Aroclor dose and pentobarbital dose increased, up to a maximum at two hours. Four hour and eight hour pretreatments with Aroclor also increased pentobarbital sleep times, but to a lesser extent. When Aroclor 1254 was administered 24 hours prior to the pentobarbital dose, the sleep time decreased. The dose-response relationship for the Aroclor-induced increase in pentobarbital sleep time was established under the conditions in which all doses of Aroclor 1254 were administered two hours before pentobarbital. Although sleep times increased for doses ranging from 5 to 500 mg/kg, the incremental increases for doses above 25 mg/kg were small. In a separate experiment, mice were treated for 14 days with either 30 or 100 mg/kg Aroclor 1254 and then challenged with a dose of pentobarbital either 45 minutes or 24 hours after the last Aroclor dose. The 14-day Aroclor treatment decreased pentobarbital sleep times, and decreases were somewhat larger for the 100 mg/kg dosage when compared with the 30 mg/kg dosage. In both cases, larger decreases in sleep time were observed when pentobarbital was administered 24 hours rather than 45 minutes after the last dose of Aroclor 1254. Additional experiments were performed to verify that Aroclor-induced changes in sleep times were the result of changes in the disposition of pentobarbital rather than some change in the sensitivity of the animal to the depressant effects of pentobarbital. Concentrations of pentobarbital and metabolites were determined in the the brain, liver, and plasma under the conditions used in the previous experiments. It was found that Aroclor pretreatment produced changes in the concentrations of pentobarbital and metabolites consistent with an inhibition of pentobarbital metabolism following acute pretreatment (up to eight hours) and induction of metabolism after subacute treatment (14 days).

3.2.4 Immunotoxicity

In early studies of PCB toxicity, atrophy of lymphoid organs was a consistent finding in several animal species (Flick et al., 1965; Vos and Beems, 1971). Subsequent studies showed that PCBs in sufficient doses could affect resistance of animals to infection. Friend and Trainer (1970) demonstrated enhanced susceptibility to viral infection of Aroclor 1254-treated animals. Ducklings fed 25, 50, or 100 ppm for 10 days had significantly higher mortality when challenged with hepatitis virus than did nonexposed controls.

Vos and de Roij (1972) evaluated the immunotoxic potential of Aroclor 1260 in guinea pigs. Three groups of four-week-old female guinea pigs, 12 to a group, were fed 0, 10, or 50 ppm Aroclor 1260 for eight weeks. The Aroclor 1260 mixture used was reported to be free of measurable dibenzofurans, but did contain some minor acnegenic contaminant. After the PCB exposure, half of the animals received a tetanus toxoid injection to stimulate the humoral response of the lymphatic system. The weight gain of the PCB-fed animals was reduced indicating that the PCB exposure used was toxic. Among unstimulated (no tetanus toxoid injection) guinea pigs, there were no observed differences in serum globulins among animals fed 0, 10, or 50 ppm PCBs. Among tetanus-toxoid stimulated guinea pigs, the percent serum protein as alpha-globulin was increased at both feeding levels of PCB, while the percent as gamma-globulin was significantly decreased only in the group fed 10 ppm PCBs. The number of gamma-globulin containing cells in the popliteal lymph nodes, visualized using a direct fluorescent antibody technique, was significantly reduced at both feeding doses of Aroclor 1260 in toxoid-stimulated guinea pigs. The numbers of pyroninophilic cells in cervical lymph nodes were decreased in stimulated animals fed 50 ppm PCBs and in unstimulated animals fed 10 ppm. However, no differences in pyroninophilic cells were detected in spleen or mesenteric lymph nodes in stimulated or unstimulated guinea pigs fed PCBs at either dose. No changes induced by PCB feeding were observed in the leukocyte counts, the number of follicles in the spleen and cervical and mesenteric lymph nodes, the number or morphology of Hassal corpuscles, or the weights of the thymus, spleen, and lymph nodes.

Vos also published a study with Dreil-Grootenhuis in 1972 that examined the effects of PCBs on the humoral and cell-mediated immune responses in guinea pigs. After feeding the animals 0, 10, 50, or 250 ppm Clophen A60, there was a suppression of tetanus-toxoid-stimulated humoral immunity in the 50 and 250 ppm treated groups. Microscopic examination of the livers revealed centrilobular necrosis. In a second experiment, guinea pigs were fed 10 or 50 ppm Clophen A60 or 50 ppm Aroclor 1260. Suppression of the humoral response was observed at 50 ppm for both PCB mixtures, as was a decrease in thymus weight.

Koller and Thigpen (1973) examined the effect of feeding rabbits three different commercial PCB mixtures (21, 42, and 54% chlorine content) on immune function. The PCB mixtures were administered by gavage once a week for 14 weeks. Rabbits were inoculated with pseudo-rabies virus, and specific antibody titers to the virus were measured by serum neutralization. At 24 days after the first inoculation, mean antibody titers were significantly lower than control for groups of rabbits treated with each of the commercial PCB mixtures. Total gamma globulin levels increased after inoculation for the controls and rabbits treated with 21 and 54% chlorine PCBs. Total gamma globulins were decreased in rabbits treated with 42% chlorine. No statistical comparisons were presented on the gamma globulin data, nor was any explanation offered as to why only one PCB mixture of intermediate chlorine content decreased gamma globulin levels.

Loose et al. (1977) examined the immune responses of male Balb/c mice fed dietary levels of 167 ppm Aroclor 1242 for six weeks. The animals were then challenged with an intravenous injection of sheep red blood cells. As measured by the splenic direct plaque-forming cell (PFC) method, PCBs reduced the antibody response by about 50%. Peak antibody response occurred at the same time in both PCB-treated and untreated mice, indicating that PCB treatment did not delay response time. PCB treatment decreased the serum concentrations of all immunoglobulins, particularly IgA. Lung, thymus, and spleen weights of the PCB-treated animals were normal and histopathological examination of these organs and the lymph nodes did not reveal any pathological changes. Liver weights were increased, an effect attributed to centrilobular and pericentral

hypertrophy. Mean tissue levels of PCBs in these animals were 2.8 ppm in liver, 7.0 ppm in lung, 2.2 ppm in spleen, 7.1 ppm in thymus, and 0.84 ppm in the blood. A second challenge with sheep red blood cells was used to determine the effects of PCBs on antibody memory cells. The number of PFCs in the PCB-treated mice was less than 50% of the control group. While IgM and IgG were unchanged, IgA was significantly lower in the animals fed PCBs in this phase of the study. The tissue PCB concentrations were similar to those observed in the first phase of the study, suggesting that the tissue concentrations had reached steady-state.

Wierda et al. (1981) assessed the PFC response of mice treated with a single intraperitoneal dose of 62, 135, or 250 mg/kg of Aroclor 1254. A significant suppression of IgM-secreting splenocytes was observed with the 135 and 250 mg/kg doses. Spleen mitogenic response to T and B cell mitogens were not affected by PCB treatment.

Loose et al. (1978) subsequently used the ability of Balb/c mice to resist endotoxin (*Salmonella typhosa* derived) or malarial infection (*Plasmodium berghei*) as functional tests of the effects of Aroclor 1242 on the immune system. As in the previous study of Loose et al. (1977), mice were fed Aroclor 1242 at a level of 167 ppm. After being fed the PCB diet for six weeks, the mice were 5.2 times more sensitive to the endotoxin as determined by the change in the LD_{50}, and there was a 20 % decrease in the survival time of the mice infected with malaria. The PCB tissue levels were the same as those reported earlier (Loose et al., 1977).

Similarly, Imanishi et al. (1980) demonstrated that ICR mice fed diets containing 100, 200, or 400 ppm Kanechlor 500 for three weeks were significantly more susceptible to the lethal effects of infection with herpes simplex virus or ectromelia virus. The inducibility of interferon in these animals, however, was not affected. The concentrations of PCBs in the livers of these animals were 230 ppm for animals fed a diet of 100 ppm PCBs and 300 ppm for animals fed either 200 or 400 ppm.

Loose et al. (1981) initiated an in-depth evaluation of macrophage function in mice fed diets of Aroclor 1242 for 3, 6, or 18 weeks, since previous studies had

suggested a macrophage defect in xenobiotic-induced immunosuppression, and because macrophages are integral components of an immune response. At a dose of 167 ppm, Aroclor 1242 was without an effect on alveolar, splenic, and peritoneal macrophages obtained from Balb/c male mice. Oxygen utilization, phagocytic activity, and microbicidal activity were unaffected by the PCB mixture. Aroclor 1242 (5 or 100 ppm for 3, 6, or 18 weeks) significantly reduced serum fibronectin concentrations, a modulator of cell surface activity, particularly at 18 weeks of dietary administration. However, there was no change in the susceptibility of the PCB-treated mice to a challenge of ascites tumor cells. Furthermore, Aroclor 1242 at 5 or 100 ppm did not alter splenic tumoricidal activity. Interestingly, adherent cell tumoricidal (mKSA tumor cells) activity was altered following Aroclor 1242 (100 ppm) administration at all time periods studied. Adherent cells from the high-dose PCB animals manifested a tumoricidal activity which was approximately 80% of control values. This reduction was not exacerbated with time on diet. The investigators concluded that the absence of any alteration in the unfractionated spleen cell tumoricidal activity in PCB-treated mice may indicate the absence of any alteration in natural killer (NK) cell activity. On the other hand, since Aroclor 1242 decreases the cytotoxic activity of adherent spleen cells against mKSA tumor cells, Loose et al. (1981) concluded that macrophages, perhaps activated *in vitro* by surface adsorption, are the target cells which are impaired in PCB-treated mice.

Several investigators have been unable to detect alterations in antibody (B-cell)-mediated immunity with commercial mixtures of PCBs (Thomas and Hinsdill, 1978). Rhesus monkeys fed 2.5 or 5 ppm of Aroclor 1248 for five months and immunized with sheep red blood cells (SRBC) showed consistently depressed IgG levels, but antibody response was reduced on only two occasions in the 5 ppm group. Antibodies to tetanus toxoid were not affected. The monkeys treated with the Aroclor showed overt signs of toxicity.

Similarly, antibody response was not suppressed in immunized offspring of rabbits fed 10, 100, or 250 ppm of Aroclor 1248 for four weeks (Thomas and Hinsdill, 1980). Offspring of dams fed 250 ppm showed some contact sensitivity, but these offspring also showed reduced body weight gains. These results are in agreement

with those of Talcott and Koller (1983), in which offspring of PCB-treated mice showed no alteration in humoral immunity (see below).

These same authors studied the susceptibility of Aroclor 1248-treated mice to infection (Thomas and Hinsdill, 1978). Mice were treated with up to 1000 ppm for three to five weeks without evidence of overt toxicity, but the Aroclor-treated animals showed a higher mortality when challenged with the pathogen *Salmonella typhimurium* than did controls.

Silkworth and Grabstein (1982) tested the immunotoxic potential of two tetrachlorobiphenyl isomers in *Ah*-responsive and nonresponsive mice. In this study, they attempted to determine whether or not immunotoxicity correlated with the *Ah*-locus and/or the planarity of the PCB isomer. Both C57BL/6 (B6; *Ah*-responsive) and DBA/2 (D2; *Ah*-unresponsive) mice were administered either 3,4,3',4'-tetrachlorobiphenyl (which assumes a planar conformation) or 2,5,2',5'-tetrachlorobiphenyl (which cannot assume a planar conformation) for two days before and after an injection of sheep red blood cells. Five days after immunization, the primary antibody response was evaluated using a PFC-assay. A dose of 100 mg/kg of the 3,4,3',4'-tetrachlorobiphenyl isomer substantially reduced the number of PFCs in the *Ah*-responsive B6 mouse. Other signs of toxicity were noted including thymic atrophy, hepatic fatty infiltration, decreased body weight, and decreased spleen weight relative to body weight. The same dose of 3,4,3',4'-tetra- chlorobiphenyl in the nonresponsive D2 mouse did not affect these parameters. A similar dose of 2,5,2',5'-tetrachlorobiphenyl had no effect on the appearance of PFCs in either strain. The authors concluded that the immunotoxicity of the PCBs is dependent on their conformation and is genetically controlled. These conclusions have been supported in more recent investigations (Silkworth et al., 1984; 1986; Lubet et al., 1986), which have further concluded that activation of the *Ah* gene complex is the initial requirement for PCB immunotoxicity.

Talcott and Koller (1983) used Swiss-Webster mice to determine if PCB exposure to female mice produced an effect on immune function in their offspring. Female Swiss-Webster mice were fed dietary Aroclor 1254 at levels of 10, 100, or 250

ppm for 12 weeks. After this exposure, the mice were bred. The exposure was continued through weaning and lactation. The offspring were weaned on a control diet at three weeks of age and the dams sacrificed. The dams suffered no weight loss as a result of PCB exposure, and no noticeable detrimental effects on reproduction were observed. Humoral immune function (as measured by antibody response to bovine serum albumin), delayed-type hypersensitivity (in response to topical oxazolone), and phagocytosis of peritoneal macrophages were unaffected in pups from PCB-treated dams when compared to pups from untreated dams.

Takagi et al. (1987) also studied the effects of pre- and post-natal exposure to PCBs on immune function in offspring. PCB exposure did not significantly alter body weight, spleen weight, or spleen cellularity. Exposure to PCBs within the dam's body was not found to affect B-cell function. Some depression of T-cell function was observed, particularly in prenatally-exposed mice, which required up to 11 weeks for recovery to control levels. The authors caution that commercial PCB mixtures such as the ones used in the study contain contaminating polychlorinated dibenzofurans which may contribute to, or account for, the observed effects on T-cell function.

Beran et al. (1983), who examined the distribution and effect of ^{14}C-labelled 2,4',5-trichlorobiphenyl, 2,4,5,2',4',5'-hexachlorobiphenyl, and 2,3,4,5,2',3',4',6'-octachlorobiphenyl in both the squirrel monkey and mice (C57BL), observed that very low or negligible uptake of these labelled chlorobiphenyls occurred in the thymus, spleen, and lymph nodes, organs reported by several authors to be suppressed by commercial PCB preparations. Beran et al. (1983) concluded, therefore, that the effect of PCBs in the lymphatic system does not seem to be related to the uptake of these compounds directly in the target cells.

Silkworth and Antrim (1985) demonstrated that although thymic atrophy occurs, it is neither an accurate nor a sensitive measure of *Ah*-receptor-mediated immunosuppression. They concluded that the functional immunosuppression caused by *Ah* receptor ligands is not necessarily a direct consequence of thymic atrophy. They further demonstrated that the differentiation of the B lymphocyte into antibody-secreting plasma cells is impaired during *Ah*-receptor-mediated

gene activation, suggesting that this may be the mechanism of PCB-induced immunotoxicity in animals.

Talcott et al. (1985) have examined the effect of dietary Aroclor 1254 exposure on natural killer (NK) cell cytotoxicity in Sprague-Dawley rats. Rats fed either 50 or 500 ppm PCB for 10 weeks showed a substantial reduction in NK cell cytotoxicity. These doses, however, did not produce overt signs of toxicity or changes in body weight. Spleen cells from untreated rats exposed *in vitro* to 20 µg/ml PCB also exhibited decreased NK cell cytotoxicity. This effect was not a consequence of a loss of NK cell viability suggesting a functional deficit. The 20 µg/ml concentration must be considered to be very near a directly cytotoxic concentration of PCB, for the authors indicated that PCB concentrations above this value caused a loss of cell viability as demonstrated by trypan blue dye exclusion. The authors proposed that impairment of NK cell function by PCBs may explain, in part, increased incidence of tumors following PCB exposure. These results are supported by the work of Exon et al. (1985).

While some studies reported measurable changes in specific parameters used as some measure of immune function, these effects have only been observed at relatively high doses. The consequences of most of these effects in terms of morbidity or mortality are unclear. Only PCB exposures approximating or greater than 100 ppm were shown to increase vulnerability to infectious agents. This would seem to be supported by the observations in chronic rat cancer bioassays, in which animals fed dietary levels of 100 ppm PCBs for a lifetime did not suffer any increase in morbidity or mortality (see Section 3.7).

3.2.5 Biochemical Changes and Other Organ Toxicities

The aforementioned toxicities are those that are generally discussed in the literature. Other reported toxic effects in animals include anorexia, gastric ulcers, bloody stools, acites, degenerative changes in the kidneys, pancreatomegaly, and alopecia. Biochemical changes, changes in lipid metabolism, and effects on the thyroid have also been observed. Some of these toxic effects in other organs and systems in animals will be discussed below.

Iatropoulos et al. (1977) administered 2,5,4'-trichlorobiphenyl in the diet to rhesus monkeys for 84 days. Some monkeys were allowed to recover for 28 days after the PCB exposure period before sacrifice. Histopathological examination of the monkeys revealed widespread, reversible changes in small vessels including arterioles, capillaries, and venules. A number of organs had venous congestion. Degenerative changes in the kidneys were noted, as well as the CNS, particularly the hippocampal area. The authors suggested that venous congestion may contribute to regional hypoxia.

Gastric hyperplasia has been observed in PCB-exposed animals, particularly monkeys. Tryphonas et al. (1984) have reported hypertrophic gastropathy among monkeys treated with either Aroclor 1254 (5 mg/kg/day, 3 days per week) or Aroclor 1248 (2 mg/kg/day, 3 days per week). Cystic dilation of gastric glands was also observed, more consistently among Aroclor 1254-treated monkeys. Gastric hyperplasia was found in subsequent studies using lower dosages of Aroclor 1254 (0.2 mg/kg/day, 5 days per week), but cystic dilation of gastric glands was seen in only 1 of 4 monkeys (Tryphonas et al., 1986a&b).

PCBs have been reported to lower tissue levels of various vitamins. One group of investigators (Bitman et al., 1972; Cecil et al., 1973) fed Japanese quail or rats of both sexes diets containing 100 ppm Aroclor 1242 for two months. Additionally, the quail and rat diets contained 10,000 and 9,120 μg vitamin A per kg feed, respectively. In rats, Aroclor 1242 increased liver weights in both males and females, although the increase in males was significantly greater than was seen in females. On the other hand, the liver concentration of vitamin A in both sexes was significantly reduced. In both male and female rats, Aroclor 1242 decreased the liver vitamin A concentration by approximately 50%. The decreased concentration of vitamin A in the liver was not due to a dilutional effect caused by the increase in liver size as the vitamin A per total liver was also significantly reduced. Interestingly, in the quail, the livers of females were twice as large as those of the male. However, while vitamin A concentrations were significantly lower (~ 50% decrease) in male quail, Aroclor 1242 had no effect on the vitamin A content in female quail. This lack of effect of Aroclor 1242 on vitamin A

concentration in the livers of female quail was attributed to the fact that vitamin A levels are normally at low levels during egg laying. Indeed, when females were kept in the dark, a condition that arrests egg laying and cyclic mobilization of vitamin A and deposition in the yolk, administration of Aroclor 1242 via the diet decreased vitamin A content in the liver by approximately 50%. The authors noted that while Aroclor 1242 reduced liver vitamin A storage by 50%, no symptoms of vitamin A deficiency were evident. They concluded, however, that in animals receiving a marginal level of vitamin A in their diet, PCBs could reduce liver vitamin A stores to such an extent that avitaminosis might occur.

Brouwer and van den Berg (1983;1984) similarly demonstrated in preliminary experiments that both Aroclor 1254 and 3,4,3',4'-tetrachlorobiphenyl reduced retinol levels in C57BL/Rij mice. They then examined the effects of 3,4,3'4'-tetrachlorobiphenyl on retinol levels in both the C57BL/Rij (*Ah*-responsive) and DBA/2 (*Ah*-nonresponsive) mice. Weekly intraperitoneal injections of 1.5 to 100 mg/kg of this PCB isomer were given for four weeks. In C57BL/Rij mice, there was a dose-dependent decrease in both liver and serum retinol, while in the DBA/2 mice there was a decrease in only serum retinol. The authors suggested that in the C57BL/Rij mice, the reduction in liver retinoids led to a concomitant decrease in serum retinol, since it has been shown that a decrease in liver retinol reduces the secretion of retinol binding protein, a specific carrier for serum retinol. The decrease in liver retinol in this strain, however, was not due to the induction of aryl hydrocarbon hydroxylase (AHH) since induction of this enzyme occurred after the reduction in liver retinol. The mechanism involved in the reduction of serum retinol in DBA/2 mice could not be explained. The investigators concluded that reduction in retinol levels could be used as a sensitive indicator of PCB intoxication since the effect of PCBs on retinol levels was more pronounced than that seen in other PCB-induced toxicities (e.g., decrease in thymus and body weight, increase in liver weight, and induction of AHH activity).

Azais et al. (1986) demonstrated that not all PCB isomers are capable of reducing vitamin A levels following systemic absorption. In their experiments, male Sprague-Dawley rats were given a single intraperitoneal injection of 300 mol/kg of 2,5,2',5'-tetrachlorobiphenyl, 3,4,3',4'-tetrachlorobiphenyl, or

2,4,5,2',4',5'-hexachlorobiphenyl, and the concentration of vitamin A in the liver was measured. Only the livers of those rats treated with 3,4,3',4'-tetrachlorobiphenyl showed significantly diminished total vitamin A levels. Although the mechanism for this decrease was unknown, the investigators did conclude that the reduction of liver vitamin A was not due to induction of various hepatic drug-metabolizing enzymes or retinyl palmitate hydrolase activities.

In a subsequent study, Brouwer et al. (1986) demonstrated that the reduction of vitamin A seen after a single intraperitoneal injection of 50 mg of 3,4,3',4'-tetrachlorobiphenyl in female mice (C57BL/Rij and DBA/2) and rats (Sprague-Dawley) was due to a direct interaction of a metabolite of this PCB isomer with transthyretin, leading to an inhibition of formation of the plasma transport protein complex carrying both retinol and thyroxin. Consequently, serum levels of retinol and its primary carrier protein, retinol binding protein, as well as serum T_4 levels are strongly reduced upon treatment of rats and mice with 3,4,3',4'-tetrachlorobiphenyl. As a result, the delivery of retinol to its target epithelia is strongly diminished, which in the end may lead to pathological changes of epithelia associated with a deficiency of vitamin A, such as hyperkeratosis. Furthermore, the altered ratios of free versus bound thyroid hormones may change the basal metabolic rate of energy conversion and may therefore accelerate lipid catabolism in exposed animals. This may explain why loss of body fat is often encountered in toxicity by PCBs.

Powers et al. (1987) have proposed that the decrease in plasma retinol levels in PCB-treated rats is due to the inhibition of hepatic retinol palmitate hydrolase, thus prohibiting the release of retinol from stored retinyl esters within the hepatic parenchymal cells. In their experiments, Powers et al. (1987) administered a single intraperitoneal injection of 1, 5, or 15 mg 3,4,3',4'-tetrachlorobiphenyl per kg body weight to female Sprague-Dawley rats. Additionally, they administered single doses of 2,4,5,2',4',5'- and 3,4,5,3',4',5'-hexachlorobiphenyl to separate groups of rats. Only 3,4,3',4'-tetrachlorobiphenyl caused a dose-dependent decrease in the activity of hepatic retinol palmitate hydrolase, a reduction that correlated ($R = 0.90$) with the reduction of plasma retinol. Furthermore, this PCB

isomer also inhibited this enzyme *in vitro*, an effect that could be overcome by an initial incubation of the microsomal-PCB mixture with an NADPH-generation system. Thus, the authors concluded that the mechanism by which 3,4,3',4'-tetrachlorobiphenyl causes lowered plasma retinol levels is the inhibition of hepatic retinyl palmitate hydrolase, and that this inhibition is due to the parent isomer itself rather than a metabolite.

Yagi et al. (1979) examined the effect of PCB (Kanechlor-500) administration on thiamine (vitamin B_1) metabolism in the rat. Male Wistar rats were fed diets containing 500 ppm Kanechlor-500 for 48 and 49 days, at which time the thiamine levels in blood, urine, feces, and various tissues were determined. These animals had depressed growth rates, increased liver weights, and increased liver to body weight ratios. Thiamine contents in blood, liver, and sciatic nerve were decreased after 49 days, while urinary, fecal, renal, and brain thiamine levels were unaffected. Interestingly, diphenyl (254 ppm) had no effect on thiamine levels. Transketolase activity in erythrocytes and liver was decreased while an increase in thiamine pyrophosphate activity in erythrocytes and liver was noted. The investigators concluded that the decrease in thiamine was not due to a decrease in intestinal metabolism since the urinary and fecal concentrations of thiamine were no different from the control. They further concluded that this decrease was the result of an increase in metabolizing enzymes, perhaps thiamine pyrophosphate, induced by PCB administration.

Finally, Ohchi et al. (1986) examined the effects of the dietary addition of 200 or 500 ppm Aroclor 1248 on tissue levels of essential trace elements, iron, zinc, copper, manganese, molybdenum, chromium, nickel, and cobalt in male Wistar rats. Following 15 days of PCB administration, both dietary levels caused increases in liver concentration of copper, and there was a significant correlation between dietary level of PCB and liver level of copper ($r = 0.99$; $p < 0.01$). The liver concentration of cobalt was also significantly increased by 200 ppm Aroclor 1248, but not at the higher dietary level. Liver concentrations of other elements such as iron, zinc, manganese, molybdenum, chromium, and nickel were not influenced by PCB intake. In the kidney, PCB administration resulted in an increase in the level of cadmium, but copper and zinc were unaffected. On the other hand, tibia

zinc was significantly increased by Aroclor 1248 while tibia iron was depressed. Serum levels of zinc and copper were also significantly increased by the dietary addition of PCB. Thus, PCBs may affect the metabolism not only of vitamins, but of mineral and trace elements as well, and these effects may be related to the toxicities exhibited by this family of compounds.

Aroclor 1254 was shown to elevate serum corticosterone in mice (Sanders et al., 1974). Mice were exposed to dietary levels of 62.5, 250, and 1000 ppm for two weeks. Adrenal weight was increased only by the 1000 ppm regimen. Whether the effect on corticosterone is a direct effect or an adaptive response to toxicity is difficult to ascertain. The 1000 ppm dose produced 60% mortality and both the 1000 and 250 ppm doses caused a significant reduction in food intake. In addition, the 62.5 ppm group showed significant body weight loss.

A few studies have examined the effect of PCBs on neurotransmitters. Seegal et al. (1985a) examined the effects of a single dose (500 or 1,000 mg/kg body weight) of a mixture containing equal weights of Aroclors 1254 and 1260 on concentrations of norepinephrine in the brains of adult male Wistar rats. The regions examined were the frontal cortex, hippocampus, hypothalamus, and brainstem. Following a single oral dose of 1,000 mg/kg of the Aroclor mixture, brain regions behaved dramatically differently from each other in terms of both initial PCB concentrations and redistribution patterns. The brainstem contained the highest concentrations of PCBs; the frontal cortex contained the lowest. Similar patterns were seen in animals that received 500 mg/kg of the PCB mixture, although the redistribution pattern observed in animals that received the higher dose was absent. Norepinephrine concentrations were reduced in the frontal cortex and hippocampus but were unaffected in the hypothalamus and brainstem. In those areas in which norepinephrine was reduced, the levels returned to normal within 20 days following PCB administration. However, it is the opinion of the reviewers that no association can be made between PCBs and norepinephrine levels in the brain. First, norepinephrine levels were not reduced in that area which contained (relatively) the highest concentration of PCBs, the brainstem. Rather, norepinephrine was reduced in the area containing the lowest level of PCBs, the frontal cortex. The authors suggested that this difference was

due to the fact that PCBs may differentially affect the synthesis of norepinephrine from nerve terminal areas rather than affecting synthesis and storage of this neurotransmitter in brain regions that have a high density of noradrenergic cell bodies. Assuming this were true, PCBs should have had an effect on norepinephrine levels in the hypothalamus which, as the authors stated, they did not. Also, the levels of norepinephrine were reduced following administration of corn oil (most likely due to stress) and the levels measured were not much different from those following PCB administration (although the authors reported them to be statistically significant). Because of the normal variance in norepinephrine levels from day to day and from animal to animal, the decrease in norepinephrine levels following administration of PCBs may only reflect differences between animals due to handling or administration technique. Furthermore, even if the decrease in norepinephrine levels in the frontal cortex, for example, were decreased as a result of the administered PCBs, the decrease was only slight (~0.63 ng/mg tissue in controls versus ~0.54 ng/mg tissue following administration of 1,000 mg/kg of the PCB mixture), and of doubtful biological consequence.

Seegal et al. (1985b) also suggested that increase in urinary homovanillic acid, the major metabolite of dopamine, brought about by a single oral dose of 1,000 mg/kg body weight of the same PCB mixture used above, indicated that PCBs cause neurochemical changes and in particular, significantly alter brain concentrations of dopamine (Seegal et al., 1986). These investigators also stated that based on these findings, urinary homovanillic acid measures reflect the neurotoxic actions of the PCBs. Again, it is the consensus of the reviewers that no association can be made between PCBs and dopamine levels in the brain. First, as the authors stated, the source of the elevated urinary homovanillic acid was not identified. Second, the amount of change seen upon administration of a massive PCB dose (1,000 mg/kg) was small (from a control of ~32 µg/24 hours to ~42 µg/24 hours) and of questionable clinical relevance despite the authors' indication that the increase was statistically significant. And finally, the increase in urinary homovanillic acid was apparent for only a very short period of time (8 to 11 hours post administration) and returned rapidly to control levels.

Heinz et al. (1980) studied the effects of exposure to PCBs, dieldrin, or DDE on dopamine and norepinephrine in the brain of ring doves. Doves were fed 0, 1, 10, or 100 ppm Aroclor 1254. The highest dosage (100 ppm) produced nearly a 50% decrease in both dopamine and norepinephrine brain levels, and the levels of these neurotransmitters were negatively correlated with residual PCBs measured in the brain. The 10 and 100 ppm dosages apparently caused generalized toxicity, as body weight losses occurred among doves fed PCBs at these levels. At 1 ppm exposure, no body weight changes or neurotransmitter decreases were observed.

As stated above, PCBs are thought to affect the thyroid, and thus thyroxine blood levels, following systemic absorption. Brouwer et al. (1986) demonstrated that administration of 3,4,3',4'-tetrachlorobiphenyl resulted in the reduction of blood T_4 levels. Studies have shown that PCBs are capable of producing ultrastructural lesions in thyroid follicular cells and reducing serum thyroxine levels in the rat. Collins et al. (1977) fed Osborne-Mendel rats diets consisting of 50 or 500 ppm Aroclor 1254 for either 4 or 12 weeks. This exposure period was followed by 12- and 35-week elimination intervals during which the rats received no PCBs prior to sacrifice. The 500 ppm diet proved too toxic after six weeks of the 12-week exposure interval, and the animals in that group had to be changed to diets containing 250 ppm for the remaining 6 weeks. After only 4 weeks of PCB exposure, the effects on the thyroid gland consisted of an accumulation of lysosomal bodies and colloid droplets in follicular cells with abnormalities of the microvilli that were present on the luminal surface. The cytoplasmic acid-phosphatase activity was also increased in follicular cells at both doses. With longer administration of PCBs there was a striking distention of many of the follicular cells, and these cells contained large lysosomes rich in acid-phosphatase activity and colloid droplets. The microvilli of the follicular cells were blunted and abnormally branched, and there were mitochondrial vacuolations. These histopathological changes were associated with a decrease in serum thyroxine levels. Twelve weeks post-exposure, the ultrastructural changes and decrease in serum thyroxine levels were still present. However, 35 weeks post-administration of the PCBs, the thyroid gland appeared normal and the thyroxine levels had returned to control values. Because the follicular cells had hypertrophied and were more columnar than in control animals, the authors felt that some of the

observed changes were due in part to a compensatory increase in secretory activity in response to the lower serum thyroxine levels. This compensatory hypertrophy and hyperplasia of the follicular cells is produced by a number of agents that induce liver metabolism thereby lowering thyroxine levels; this ultimately causes the thyroid gland to increase thyroxine production (Fregly et al., 1968; Collins et al., 1977; Bastomsky, 1974; Hurst et al., 1974). Thus, it was concluded that the PCB-induced lowering of thyroxine levels not only explained some of the observed ultrastructural changes in the thyroid gland, but that this "hypothyrodism" might be the direct cause of other PCB-induced toxicities such as a decrease in weight gain, skin lesions, hyperpigmentation, and a decreased reproductive performance (Collins et al., 1977).

In a subsequent study, Collins and Capen (1980c) fed female Osborne-Mendel rats diets containing 50 or 500 ppm Aroclor 1254 during pregnancy and apparently for up to 21 days post-parturition. Thyroid glands were collected for examination from the neonates and dams on day 18 of gestation, at parturition, and from the neonates at 7, 14, and 21 days of age. Changes in thyroid ultrastructure and reductions in serum thyroxine were similar to those they had previously reported for adult animals (Collins et al., 1977; Collins and Capen, 1980a&b) and were observed in the pups, indicating that the effects of PCBs on the thyroid gland and hormone might occur in exposed newborns.

Sepkovic and Byrne (1984) examined triiodothyronine kinetics in rats treated with PCBs. After seven months of treatment with either Aroclor 1242 or Aroclor 1254, animals in both PCB-treated groups had serum triiodothyronine levels lower than control. Interestingly, the administration of radiolabelled triiodothyronine revealed a slower degradation rate for rats treated with Aroclor 1254 (but not Aroclor 1242), though the magnitude of the change was not striking (slope of serum triiodothyronine level change, in %/hr, was 0.014 for controls and 0.011 for Aroclor 1254-treated rats).

Bryne et al. (1987) expanded their studies of the effects of PCBs on thyroid hormone kinetics in rats. Sprague-Dawley rats were fed 0, 1, 5, or 50 ppm Aroclor 1254 for five to seven months. Serum T_3 and T_4 levels were suppressed in a

dose-related manner by PCB treatment. A thyroid stimulating hormone (TSH) injection showed a diminished response in treated rats, and T_4 production rates were decreased. Monitoring of injected radiolabelled T_4 showed an 8-fold increase in T_4 distribution volume in PCB-treated rats, but body weights and thyroid weights were not significantly altered. The authors concluded that PCB treatment-induced suppression of T_3 and T_4 did not appear to result from effects on the hypothalamic-pituitary axis and was primarily the result of direct damage to the thyroid rather than enhanced hepatic or other peripheral catabolism. Byrne proposed that histological and ultrastructural cell damage caused by PCB/PBB exposure expanded pools for T_4 dilution, and that TSH plays little role in the induced hypothyroidism. These conclusions are largely speculative, however, because neither histological nor ultrastructual changes were demonstrated by the authors for the PCB dose levels administered, nor was it shown that an increased T_3 and T_4 catabolism peripherally could be reasonably ruled out.

Disturbances of cholesterol metabolism have also been noted by a number of investigators. In one study, PCBs increased the synthesis of cholesterol in rats fed a diet containing 500 ppm Kanechlor-500 for 35 days (Yagi and Itokawa, 1980). In another study, Fisher rats were fed diets containing 0, 2, 4, 8, 16, or 32 ppm Aroclor 1254 for four days (Carter, 1984). Serum total cholesterol concentrations were significantly elevated in the rats fed 8, 16, or 32 ppm of the PCB mixture. While there was a tendency for the high density lipoprotein-cholesterol (HDL) values for all PCB-treated rats to be greater than controls, only the differences for rats fed 32 ppm were statistically significant. This same investigator performed a more detailed analysis of lipoprotein changes induced by PCBs in a subsequent study (Carter, 1985). Fisher rats were also used in this study, and the PCB exposure levels were 0, 4, 8, and 16 ppm of Aroclor 1254 in the diet for four days. As in the previous study, total serum cholesterol concentrations were elevated at the 8 and 16 ppm exposures. HDL-cholesterol was also elevated at these exposure levels. Low-density lipoprotein (LDL)-cholesterol and very-low-density lipoprotein (VLDL)-cholesterol were unchanged, however.

Hladka et al. (1983) measured serum lipids in rats administered doses of a

commercial PCB mixture up to 50 mg/kg/day, and found increases in cholesterol and total serum lipids, and modest increases in serum triglycerides. Quazi et al. (1983 a&b) looked at the effect on lipids of 300 ppm Aroclor 1248 in the diet of rats for three weeks. Male and female rats of the Donryu, Wistar, and Sprague-Dawley strains all had higher total serum cholesterol and HDL-cholesterol concentrations after this exposure to PCBs. Serum LDL- and VLDL-cholesterol were reported combined in this study. While there was a trend to higher LDL-VLDL concentrations in PCB-treated animals, only two groups (Donyru females and Wistar males) were significantly different from controls. Serum triglyceride levels were significantly elevated in all PCB-exposed groups. Quazi et al. (1984b) later suggested that this increase in cholesterol may be due to increased synthesis of cholesterol relative to degradation rate. The effects of PCBs on lipid and phospholipid metabolism are complex (e.g. Dzogbefia and Gamble, 1986), and studies *in vitro* suggest that the influence of PCBs on lipid metabolism may be direct (Rogers et al., 1983; Beranek et al., 1984). However, Nagaoka and coworkers have observed that the hypercholesterolemia produced by PCBs in Wistar rats could be blocked by the alpha adrenergic receptor blocking drug phentolamine (Nagaoka et al., 1986). This, in turn, suggests that the apparent elevation in cholesterol in rats may be secondary to PCB effects on catecholamines, and increased serum catecholamines were in fact observed in PCB-treated rats.

A recent study by Oda et al. (1987) expanded on previous work studying the relationship between PCB exposure, serum cholesterol, and urinary excretion of ascorbic acid (Quazi et al., 1984a; Oda et al., 1986). The PCB-induced elevation of serum cholesterol in rats was used to study the effects of changes in dietary cholesterol and sulfhydryl amino acids on serum cholesterol levels and urinary excretion of ascorbic acid. Male Wistar rats were fed diets consisting of 0.02% Aroclor 1248 and 10-20% casein (high cholesterol) or soy protein (low cholesterol) with or without either L-methionine or L-cystine (0.5%) for up to 21 days. Methionine increased serum cholesterol and urinary ascorbic acid in PCB-fed rats receiving low or high cholesterol diets compared to controls on parallel diets without sulfhydryl supplement. Cystine supplement appeared to increase liver weights, liver cholesterol, and serum cholesterol in a dose-dependent fashion in PCB-fed rats. Oda et al. (1987) concluded that methionine play an important role

in serum cholesterol levels, and that total sulfur-containing amino acids plays an important role in urinary excretion of ascorbic acid. In another recent study using a strain of rat unable to synthesize ascorbic acid, this research group found that ascorbic acid did not appear to influence serum cholesterol effects of PCBs, but was associated with elevated liver cholesterol (Horio et al., 1987).

PCBs appear to have a number of effects on cellular metabolism within the body. Narbonne (1979) found that Sprague-Dawley rats exposed to 10 ppm Phenoclor DP6 had an increased rate incorporation of radiolabelled amino acid into microsomal protein. This finding is not surprising in light of the well-documented proliferation of smooth endoplasmic reticulum which appears rapidly after even small doses of PCBs. A number of reports have indicated effects on mitochondrial respiration. Khan and Cutkomp (1982), using mitochondria derived from the cockroach (*Periplaneta americana*), found a concentration-dependent uncoupling of oxidative phosphorylation by PCBs.

Nishihara (1985) studied the effects of biphenyl and commercial PCB mixtures (Kanechlors) on rat mitochondrial function. Kanechlor 400 was found to inhibit the mitochondrial electron transport chain and uncouple oxidative phosporylation through a non-classical means (Nishihara, 1983). Among Kanechlors of increasing chlorination, the ability to uncouple oxidative phosporylation, as indicated by stimulation of stage 4 respiration, was Kanechlor 300 > Kanechlor 400 > Kanechlor 500. Kanechlor 600 had no effect. Shortly after this study, Nishihara reported that the uncoupling effect of Kanechlor 400 was due to a non-specific increase in the permeability of mitochondrial membranes to ions (Nishihara, 1984). Kanechlor-400 was later found to cause an increased permeability of the mitochondrial inner membrane (Nishihara, 1985), an effect shared by biphenyl. Biphenyl was more rapid than Kanechlor-400 in producing this effect. The increased permeability caused a dissipation of mitochondrial membrane potential, and both the biphenyl and the PCB mixture produced an uncoupling of oxidative phosphorylation. When the study of chlorinated biphenyls was expanded, it was found that the ability to stimulate the release of potassium ion from mitochondria, causing dissipation of mitochondrial membrane potential, occurred in the following order: biphenyl > Kanechlor 200 > Kanechlor 300 >

Kanechlor 400 > Kanechlor 500 > Kanechlor 600 (Nishihara and Utsumi, 1985).

It has recently been suggested that hydroxylated metabolites of PCBs may mediate some toxic effects by uncoupling mitochondrial respiration (Ebner and Braselton, 1987). Hydroxylated metabolites of certain PCB congeners were shown to induce mitochondrial swelling, stimulate mitochondria respiration, and uncouple mitochondrial respiration *in vitro*. A hydroxyl group was required to produce these effects on mitochondria, and efficacy was increased with the increasing chlorination; coplanarity was not a determining factor. Whether these effects are significant *in vivo* has yet to be determined.

Disruption of oxidative phosphorylation, and particularly disruption of mitochondial calcium homeostasis, have been suggested to be involved in the hepatotoxic effects of PCBs (Nishihara and Utsumi, 1986). The ability of PCBs to uncouple oxidative phosphorylation may also explain, in part, the observation of Lee and Park (1979), who reported a decreased response to mitogen (phytohemagglutinin) stimulation by human lymphocytes *in vitro* when exposed to 10^{-5} M Aroclor 1254. The authors attributed this decreased responsiveness to diminished intracellular ATP in the lymphocytes. Lee and Park (1980) also reported that 10 μM concentrations of PCBs *in vitro* decreased glucose uptake in leukocytes. The results of these *in vitro* studies must be interpreted with caution, however. Sharom and Mellors (1980) reported that PCBs were toxic to mouse lymphocytes at 10^{-5} to 10^{-6} M, and found that PCBs at these concentrations produced nonspecific toxic effects from membrane perturbations due to their high lipophilicity.

Membrane effects of PCBs are also illustrated by the study of Byrne and Sepkovic (1987) who examined monovalent cation transport in erythroid cells. Cells were derived from rats treated with either Aroclor 1242 or Aroclor 1254 at 50 ppm for 7 months. Uptake of rubidium ion was depressed in cells from Aroclor 1254-treated rats, but not rats treated with Aroclor 1242 or controls.

PCBs have also been observed to produce alterations in porphyrin metabolism leading to porphyria. Goldstein et al. (1974) found that PCBs can induce porphyria

in female Sherman rats fed 100 ppm Aroclor 1254. This porphyria was characterized by a delayed onset (two to seven months), the excretion of large amounts of 7- and 8-carboxyporphyrin in the urine, and the accumulation of uroporphyrins in the liver. Delta-aminolevulinic acid (ALA) synthetase was induced, but this effect appeared after the first evidence of disturbed porphyrin metabolism. PCB-induced porphyria was different from that induced by the classical porphyrogenic compound allyl-isopropylacetamide in that cytochrome P-450 concentrations were increased instead of decreased. Goldstein et al. (1976) also found that several hexachlorobiphenyls caused the accumulation of uroporphyrin and induction of ALA synthetase in chicks. Honda et al. (1983) observed that rats treated with Kanechlor 400 for 43 weeks had increases in liver content of both protoporphyrin and coproporphyrin, and Harada et al. (1986) found that fasting increased the porphyria from PCB exposure in rabbits.

De Verneuil and coworkers used the cultured chick hepatocyte to study potential mechanisms of PCB-induced porphyria (De Verneuil et al., 1983). In these hepatocytes, Aroclor 1254 caused an increase in ALA synthetase activity and uroporphyrin accumulation. A decrease in uroporphyrinogen decarboxylas activity was also noted, supporting the contention that PCB effects on porphyrin metabolism are due to inhibition of this enzyme.

In a subsequent study of PCB-induced porphyria, Sano (1985) examined the porphyrin-inducing effects of a variety of PCBs in cultured chick liver cells and the inhibitory effects of these isomers on uroporphyrinogen decarboxylase activity. The accumulation of uroporphyrin was highly congener-specific, and only the 3,4,3',4'-tetrachlorobiphenyl and the 3,4,5,3',4',5'-hexachlorobiphenyl isomers produce a marked accumulation. These isomers also strongly inhibited uroporphyrinogen decarboxylase. Rifkind et al. (1985) also found the effect on uroporphyrin decarboxylase in chick embryos to be highly dependent upon the congener examined.

Sinclair et al. (1986) found that the accumulation of uroporphyrin by chick embryo hepatocytes in response to treatment with 3,3',4,4'-tetrachlorobiphenyl could be reversed with piperonyl butoxide treatment. Other methylenedioxyphenyl

compounds also decreased this accumulation. While these compounds possess the ability to decrease cytochrome P-450 mediated metabolism, the cytochrome P-450 inhibitor SKF 525A did not block PCB-induced uroporphyrin accumulation. The authors concluded that the inhibitory effects of piperonyl butoxide on uroporphyrin accumulation were not due to an effect on cytochrome P-450 metabolism, and further proposed that inhibition of uroporphyrin decarboxylase by PCB congeners was not due to the formation of a reactive metabolite which covalently binds and inactivates the enzyme as had been suggested by some authors.

3.2.6 Summary of Acute, Subchronic, and Chronic Effects

The acute toxicity of PCBs is sufficiently low as to be described as "slightly toxic" or "practically non-toxic" by American Industrial Hygiene Association standards. A number of toxic effects are observed with subchronic exposure, principally involving the skin, liver, and immune system. While dermal effects of PCBs may manifest themselves somewhat differently among species, they are generally the result of hyperplasia and hyperkeratosis. In the monkey, like man, acne-like lesions may be formed.

Liver effects of PCBs observed in animals include hypertrophy, lipid vacuolization (fatty liver), and necrosis. Certain liver effects appear to be reversible with time. Necrosis appears to be isomer-dependent. PCBs also produce enzyme induction in the liver and other organs. A variety of enzymes are affected, including cytochrome P-450. Specific PCB congeners generally produce a pattern of cytochrome P-450 isozyme induction which resembles that produced by either phenobarbital or 3-methylcholanthrene. Commercial mixtures contain both types of congeners and produce induction resembling both phenobarbital and 3-methylcholanthrene.

A variety of immune abnormalities have been observed following PCB administration which in general indicate a decreased functional capability. The immune system does not seem to be especially sensitive to PCBs, and immune effects are primarily observed at PCB doses producing toxicity to other organs. PCB

administration has also been found to cause decreases in hepatic retinol and serum thyroxine levels, altered lipid metabolism leading to elevations in serum levels of some forms of cholesterol and triglycerides, and alterations in porphyrin metabolism leading to porphyria. The extent to which any of these changes is related to morbidity with PCB administration has not been established, however.

A summary of the results of individual acute and subchronic PCB toxicity studies appears in Table 3.2.5.

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 21% Chlorine PCB Mixtures			
Species not reported	M/ (10)	6 mos.	"PCBs-1221"/ethyl alcohol	250 mg/L in drinking water for 10 weeks	Elevated plasma corticosterone; hyperactivity of zona fasciculate of adrenal cortex.	Wasserman et al., 1973
Balb/C mouse	M/ (25/group)	NA	Aroclor 1221/diet	0, 3.75, 37.5 or 375 ppm for 6 months/ 6 months	Aroclor 1221: no liver lesions.	Koller, 1977
Wistar rat	M/ (NR)	young	Aroclor 1221/ Peanut-oil/i.p.	50 mg/kg/day for 3 days	Induction of the following hepatic enzymes: aniline hydroxylase, carboxylesterase, bromosulfopthalein (BSP)-glutathione "conjugase" and p-nitrophenol-glucuronic acid "conjugase."	Ecobichon 1975
New Zealand rabbit	M, F (20/group)	NR	Aroclor 1221/ corn-oil/oral	0 or 300 mg 1 time weekly for 14 weeks	No-effects on serum enzymes or liver pathology in Aroclor 1221-exposed animals.	Koller and Zinkl, 1973

*M=Male, F=Female, NR=not reported, NA= not applicable

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of ~41% Chlorine PCB Mixtures			
C57BL/6 mouse	M/ (NR)	18-20 g	Aroclor 1016/diet	167 ppm for 3 weeks	Splenic cells from treated mice injected into neonates elicited greater graft vs. host response indicating Aroclor 1016 may activate donor lymphocytes.	Silkworth andLoose, 1978
Sherman rat	M/ (27/group 52/group)	NA	Aroclor 1016/diet	100 ppm for 10 months	Levels of PCB-derived components in adipose tissue reached a level of 82 ppm (1016) after two months of exposure. No overt clinical symptoms of poisoning. At autopsy, organs appeared grossly normal. Liver weights of Aroclor 1016 animals did not differ from controls. Changes of the livers included enlarged cells with vacuolated cytoplasm and inclusions.	Burse et al.,1974
Wistar rat	M/ (NR)	young	Aroclor 1016/Peanut-oil/i.p.	50 mg/kg/day for 3 days	Induction of the following hepatic enzymes: o-demethylase, aniline hydroxylase, carboxylesterase, bromosulfopthalein (BSP)-glutathione "conjugase."	Ecobichon 1975

*M=Male, F=Female, NR=not reported, NA= not applicable

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Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
Studies of ~41% Chlorine PCB Mixtures						
Mink and European Ferrets	M,F/ (5/group)	900-1500g	Aroclor 1016/diet	20 ppm for 28 days	Weak induction of the mixed function oxidase system. Aroclor 1242 was a more potent inducer of P-450 than Aroclor 1016 and was a more potent inducer in the male than the female. Greater 3-MC-type induction was demonstrated in ferrets compared to mink.	Shull et al., 1982
Rhesus monkey	F/ (24)	NA	Aroclor 1016/diet	0.025, 0.25 or 1.0 ppm/ NR	No abnormalities of clinical, gross or reproductive parameters in adults. 1.0 ppm: Infants born in the group were significantly smaller than controls.	Barsotti and van Miller, 1984.
Studies of 42% Chlorine PCB Mixtures						
DD mouse	M/ (114)	NA	Kanechlor 300/diet	0,100, 250 or 500 ppm for 32 weeks/32 weeks	Hepatomegaly in all treatment groups. Final body weight significantly decreased. Hepatocellular hypertrophy in all exposed groups except low-dose Kanechlor 300.	Ito et al., 1973a
Balb/CJ mouse	NR/ (4-10)	NR	Aroclor 1242/corn-oil/i.p.	1000 mg/kg bw single dose	Splenomegaly: significant by day 6, peak by day 9, gone by day 13. Cellularity: significant reduction in splenic lymphocytes day 6-10.	Carter and Clancy. 1980

*M=Male, F=Female, NR=not reported, NA= not applicable

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 42% Chlorine PCB Mixtures			
Balb/C mouse	M/ (25/group)	NA	Aroclor 1242/diet	0, 3.75, 37.5 or 375 ppm for 6 months	375 ppm 1242: significant ($p<0.01$) increase in liver weight after 6 months. MLV increased the severity of hepatic lesions. Histologically, the livers of the 375 ppm 1242 group returned to normal. 37.5 and 3.75 ppm 1242 dose had no effect on liver weight. Aroclor 1242 : 375 ppm: moderate hepatopathology.	Koller, 1977
Balb/c mouse	M/ (15/group)	18-20 g	Aroclor 1242/diet	167 ppm for 6 weeks	Antibody synthesis to antigen (sheep RBC) was significantly depressed. Control animals had an approximate two-fold increase in antibody formation over the treated mice. Serum IgA concentrations were consistently lower than controls. Gram-negative endotoxin (*Salmonella typhosa*) sensitivity was increased 5.2-32-fold. Decreased resistance to a malaria challenge was also found. A 20% decrease in mean survival time of mice fed 1242 for 3 or 6 weeks and inoculated with *Plasmodium berghei* was observed.	Loose et al., 1978b

*M=Male, F=Female, NR=not reported, NA= not applicable

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 42% Chlorine PCB Mixtures			
Balb/C mouse	M/ (15/group)	18-20 g	Aroclor 1242/diet	167 ppm for 6 weeks	Increased ($p<0.05$) sensitivity to S. *typhosa* endotoxin. Further, a 20% decrease in mean survival time of mice fed 1242 for 3 or 6 weeks and inoculated with malaria. No histopathological changes in lung, thymus, mesenteric lymph nodes, spleen. Histopathological exam of liver revealed hepatocyte hyperplasia.	Loose et al., 1978a
Wistar rat	M/ (NR)	young	Aroclor 1242/ Peanut-oil/i.p.	50 mg/kg/day for 3 days	Induction of the following hepatic enzymes: o-demethylase, aniline hydroxylase, carboxylesterase, bromosulfopthalein (BSP)-glutathione "conjugase," and p-nitrophenol-glucuronic acid "conjugase."	Ecobichon 1975
NR/rat	NR/ (4)	NR	NR; ~42% Cl/none/s.c.	10 doses on al. days and 27 doses on alt days to yield total doses of 690-1863 mg	Unusual round or oval intracellular bodies were observed in the livers. Bodies were granular, reticular, or foamy in appearance.	Miller, 1944

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 42% Chlorine PCB Mixtures			
NR/rat	NR/ (30)	NR	NR; ~42% Cl/none/s.c.	69 mg, single dose;	Fatty liver degeneration; splenic hypertrophy, local injection fibrous encapsulation	Miller, 1944
Sprague-Dawley rat	M/ (13)	200-300 g	Aroclor 1242/peanut-oil/oral/i.p.	100 - >500 mg/kg single dose. 100 mg/kg bw every other day for 3 weeks	Oral 14-day LD_{50} was 4.25 g/kg. Major toxic signs included diarrhea, body weight loss. Histopathological alterations were evident in the liver and kidneys manifested as sudanophilic vacuolation. 100 mg/kg dose every other day for 3 wks: similar histopathological changes but no overt signs of toxicity. Single 100 mg/kg dose increased liver weight and induced cytochrome P-450.	Bruckner et al., 1973
Rats, dogs chickens	NR	NR	Aroclor 1242/diet	1, 10, and 100 ppm rats and dogs 18 mos. Duration of rat and chicken reproduct-ion study not known.	After 18 months in rats, Aroclor 1242 produced no effects. Dogs fed 1242 have shown no adverse effect. Decreased survival of pups was found at 100 ppm Aroclor 1242 and decreased mating indices. No adverse effects at 1 or 10 ppm. In chickens, weight loss, decreased egg shell thickness and poor hatchability was found at 10 or 100 ppm of 1242.	Keplinger et al., 1971 (abstract)

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 42% Chlorine PCB Mixtures			
Sprague-Dawley rat	M/ (24)	200-300 g	Aroclor 1242/peanut-oil/i.p.	0, 1, 5, 25, 50 or 100 mg/kg or 100 mg/kg twice weekly for 6 weeks, 1 weekly for 4 wks.	Subacute studies: loss of body weight, hepatic and renal damage, reduced erythrocyte count, diameter and hemoglobin content, elevation in serum iron; diminished plasma corticosteroid and glucose concentrations, increased urinary excretion of protein, sugars, and coproporphyrin. Induction studies: Elevated hydroxylation, N-demethylation, cytochrome P-450 activity. Minimum effective dose for induction of hydroxylation activity was 5 mg/kg.	Bruckner et al., 1974a
	M/ (6/ treated group) (3/control group)	NR	Aroclor 1242/peanut-oil/i.p.	1 or 100 mg/kg bw single dose	Examinations of microsomal enzyme induction at 1, 5, 10, 20, 40 days post-treatment indicated maximum induction at 20 days. Hydroxylation most dramatically elevated.	Bruckner et al., 1974a
Long-Evans rat	M/ (12)	130 g	Aroclor 1242/diet	0 or 100 ppm for 4 weeks	Hepatomegaly (4.46% of bw), obvious increase in SER.	Allen et al., 1975

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 42% Chlorine PCB Mixtures			
Sprague-Dawley rat	M/ (6/group)	NA	Aroclor 1242/diet	0, 5 or 25 ppm for 2,4 or 6 months/2,4 or 6 months	Elevated hepatic microsomal enzyme activity, lipid content. Elevated urinary coproporphyrin levels. Present after 2 months at 5 ppm.	Bruckner et al., 1974b
Wistar rat	M/ (290)	NA	Kanechlor 300/diet	0, 100, 500 or 1000 ppm for 28-52 weeks/1 year	Heavy mortality. Hepatomegaly, oval cell and bile duct proliferation, fatty liver infiltration. Cholangiofibrosis at 1000 ppm level of all three Kanechlors, nodular hyperplasia. Depressed final body weight. No hepatocellular carcinoma. No remarkable changes were seen in other organs.	Ito et al., 1974
Sprague-Dawley rat	F/ (6/group)	NA	Aroclor 1242/diet	0, 75 or 150 ppm for 8 or 36 weeks/36 weeks	Both levels: marked focal necrosis and regeneration, enlarged hepatocytes; many mitoses and multinucleate cells, accumulation of pigment adjacent to veins, heaviest in Kupffer cells; accumulation of lipid droplets in cytoplasm, some with areas suggestive of lipid-cholesterol complexes; marked SER proliferation; deposits of iron; granular degeneration of mitochondria; many hepatocytes contained whorl-like membranous bodies.	Jonsson et al., 1981

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 42% Chlorine PCB Mixtures			
Sherman rat	M/ (27-52/group)	NA	Aroclor 1242/diet	100 ppm /10 months	Levels of PCB-derived components in adipose tissue reached a level of 92 ppm (1242) and of 82 ppm (1016) after two months of exposure. No overt clinical symptoms of poisoning. At autopsy, organs appeared grossly normal. Mild changes in the livers of animals fed either 1242. Changes of the livers included enlarged cells with vacuolated cytoplasm and inclusions.	Burse et al., 1974
NR/Guinea pig, rats, rabbits	NR/ (54)	NR	NR; 42% Cl/none/s.c.	69-690 mg, single dose	Local injection necrosis to fibrous encapsulation. Changes to cell layers adjacent to the site were essentially chloracne. Hepatic: centrilobular necrosis, atrophy; fatty infiltration, splenic lymphoid hyperplasia, pulmonary congestion, mortality.	Miller, 1944
Guinea pig	NR/ (10)	NR	NR; ~42% Cl/ mineral-oil/s.c.	345 mg, single dose	As above; complete mortality 13 days; pulmonary congestion more severe.	Miller, 1944
Guinea pig	NR/ (11)	NR	NR; 42% Cl/none/ dermal	34.5 mg/day for 11 days	Death within 21 days. (dermal epithelial destruction). Lesions in internal organs as above.	Miller, 1944

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 42% Chlorine PCB Mixtures			
Guinea pig	NR/ (16)	NR	NR; 42% Cl/ mineral-oil/dermal	3.5-17 mg/day for 7 or 15 days	Death within 21 days. (Dermal epithelial destruction. Lesions in internal organs as above).	Miller, 1944
NR/rabbit	NR/ (3)	NR	NR; 42% Cl/none/s.c.	690 or 1380 mg, single dose	Death in 14-72 days as above except liver contained fine droplets of fat.	Miller, 1944
	NR/ (3)	NR	NR;~42% Cl/ mineral-oil/s.c.	345 or 690 mg, single dose	Death in 42-360 days as above except liver contained fine droplets of fat.	Miller, 1944
New Zealand rabbit	M, F/ (20/group)	NR	Aroclor 1242/ corn-oil/oral	0 or 300 mg 1 time weekly for 14 weeks	Aroclor 1242: elevated SGOT, SGPT in males only. No differences in hematologic parameters, BUN, serum protein fractions. Hepatomegaly. Histopathologic, Aroclor 1242: vacuolated and granular enlarged hepatocytes, centrilobular necrosis and fibrosis.	Koller and Zinkl, 1973.
NR/ ferret	M,F/ (3/group)	10 wk	Aroclor 1242/acetone/ diet	20 ppm, daily for 266 days	Developed elongated, thickened and deformed toenails. Excessive nail growth was more conspicuous in the males than females. Hyperkeratosis at the junction of the skin and eponychium and dysplasia at the root of the nail and matrix.	Bleavins et al., 1982

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 42% Chlorine PCB Mixtures			
Mink and European Ferrets	M, F/ (5/group)	900-1500g	Aroclor 1242/diet	20 ppm for 28 days	Weak induction of the mixed function oxidase system. Aroclor 1242 was a more potent inducer of P-450 than Aroclor 1016 and was a more potent inducer in the male than the female. Aroclor 1242 caused toxicity in mink but not in ferrets. Greater 3-MC-type induction was demonstrated in ferrets compared to mink.	Shull et al., 1982
Pastel Mink	M,F/ (105)	NA	Aroclor 1242/diet	0, 10, 20 or 40 ppm /8 months	Aroclor 1242 at 5 or 10 ppm: complete reproductive failure. Mortality of all mink on ≥20 ppm.	Bleavins et al., 1980
Mink	F/ (7-8/group)	NA	Aroclor 1242/diet	0 or 2 ppm/10 months	Aroclor 1254: interference with reproduction. All Aroclors tested: no significant differences in body weight gain, hemoglobin, PCV. Aroclor 1016 (200 ppm): no effect on reproductive parameters, kit growth, and adult and kit mortality.	Aulerich and Ringer, 1977
Rhesus monkey	M/ (4-5/group)	NA	Aroclor 1242 mg/kg	1 ppm for 133 days/~190 days	No evidence of toxicity.	McNulty et al., 1980

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 42% Chlorine PCB Mixtures			
Rhesus monkey	M/ (6)	NA	Aroclor 1242/diet	0, 3, 10, 30 or 100 ppm/up to 245 days	All PCB-exposed monkeys: palpebral swelling erythema; weight loss, rough hair coat, reduced Hb, leukocytosis. Mortality of 4/6 by day 245. Gastric lesions: hypertrophic gastric mucosa consisting of elongated hyperplastic glands, destruction of parietal and zymogenic cells. Only specific region along greater curvature affected.	Becker et al.,1979
			Studies of 48% Chlorine PCB Mixtures			
Outbred albino mouse	F/ (7/group)	4-6 weeks	Aroclor 1248/diet	0, 50, 100, 500, 1000 ppm for 3-5 weeks	Mice fed up to 1000 ppm exhibited no signs of PCB intoxication other than live hypertrophy. However, mice challenged with *Salmonella typhimurium* showed higher mortality and increased sensitivity to endotoxin.	Thomas and Hinsdill, 1978
DD/ mouse	M/ (114)	NA	Kanechlor 400/diet	0,100, 250 or 500 ppm for 32 weeks/32 weeks	Hepatomegaly in all treatment groups. Final body weight significantly decreased. Oval cell formation and bile duct proliferation at mid- and high dose levels of Kanechlor 400. Hepatocellular hypertrophy. Amyloidosis in all exposed groups except high-dose Kanechlor 400groups: greater incidence associated with lower doses of lower chlorinated Kanechlors.	Ito et al., 1973a

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 48% Chlorine PCB Mixtures			
Sprague Dawley rat	M/ (4/group)	100 g	Aroclor 1248/ diet	0 or 1000 ppm up to 6 weeks	Reduced rate of weight gain: Aroclor 1248>1254> 1260. Moderate elevation of Hb, PCV. Relative neutrophilia, lymphocytopenia. Enlarged liver, decreased thymus, fatty liver degenerations, cystic areas and focal necrosis with infiltration of inflammatory cells ER proliferation, vesiculation of RER, increased number of lysosomes. Increased hepatic protein, RNA, phospholipid; decreased DNA, cholesterol. Induction of N-demethylase, nitroreductase.	Allen and Abraham-son, 1973
Wistar rat	M/ (NR)	young	Aroclor 1248/ Peanut-oil/i.p.	50 mg/kg/day for 3 days	Induction of the following hepatic enzymes: o-demethylase, aniline hydroxylase, carboxylesterase, bromosulfopthalein (BSP)-glutathione "conjugase," and p-nitrophenol-glucuronic acid "conjugase."	Ecobichon 1975
Wistar rat	M/ (6/group)	40-50g	Aroclor 1248/diet	200 or 500 ppm for 15 days	Dietary exposure to Aroclor 1248 raised copper levels in the liver but several other essential trace elements were unaffected. Increased kidney and serum copper levels were also found.	Ohchi et al., 1986

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 48% Chlorine PCB Mixtures			
Wistar rat	M/ (290)	NA	Kanechlor 400/diet	0, 100, 500 or 1000 ppm for 28-52 weeks/1 year	Heavy mortality. Hepatomegaly, oval cell and bile duct proliferation, fatty liver infiltration. Cholangiofibrosis at 1000 ppm level of all three Kanechlors, nodular hyperplasia. Depressed final body weight. No hepatocellular carcinoma. No remarkable changes were seen in other organs.	Ito et al., 1974
Sprague-Dawley rat	M/ (25/group)	NA	Aroclor 1248/diet	0 or 100 ppm for 52 weeks/65 weeks	Liver homogenates had an increased hepatic protein, RNA and lipid content; decreased DNA. Increased microsomal total protein and cytochrome P-450. Induced N-demethylase, nitroreductase. Inhibited glucose 6-phosphatase.	Allen and Abraham-son,1979
Donryu rat	M,F/ (15/group)	NA	Kanechlor 400/diet	Total intake 450-1500 ppm over 159-560 days/560 days	All treated rats: fatty liver degeneration. Females 1200-1500 mg: multiple adenomatous nodules. All rats >700 mg: liver hypertrophy. Lung abscesses pneumonia, spleen atrophy and intracranial abscesses were found frequently.	Kimura and Baba, 1973

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 48% Chlorine PCB Mixtures			
Sprague-Dawley rat	M/ (96)	NA	Aroclor 1248/diet	0 or 100 ppm for 52 weeks/65 weeks	Normal appetites, appearance, weight gain, Hb, PCV, WBC, serum protein, A/G ratios. Elevated serum total lipids, cholesterol. Cholesterol levels persisted at 65 weeks (13 weeks off exposure). Triglyceride levels fell less than controls by 65 weeks. Hepatomegaly: focal degeneration and necrosis by 13 weeks. High PCB levels were found in tissues after PCB dosing had been discontinued.	Allen et al., 1976
Rhesus monkey	F/ (12)	5.6 kg/7-10 years	Aroclor 1248/diet	0, 25 ppm for 2 months · total intake of 260-450 mg	Alopecia, edema of the lips, eyelids, face pustules involving hair follicles, pruitis. Necropsy of the animal consuming 450 mg: severe weight loss, subcutaneous edema, acute hyperplastic gastritis with focal hemorrhage, ulceration. Liver: focal necrosis, enlarged hepatocytes, lipid accumulation. Hypocellularity of bone marrow.	Allen et al., 1974b
Rhesus monkey	F/ (24), M/ (NR)	NA	Aroclor 1248/diet	0, 2.5 or 5.0 ppm for ~18 months/~3 9.6 mos.	Males (5.0 ppm level only): moderate erythema and periorbital edema. Females: more severe skin lesions (alopecia, acne); extreme weight loss, irregular menstrual cycle length, depressed serum progesterone. Considerable improvement after 1-year recovery period.	Barsotti and Allen, 1975

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 48% Chlorine PCB Mixtures			
Rhesus monkey	NR	adult	Aroclor 1248/diet	100 or 300 ppm for 2-3 months	High morbidity and mortality approaching 100% within 3 months. Gradual weight loss, alopecia lacrimations, conjunctival congestion, facial edema, comedones, large intrafollicular keratin cysts. Hematology: gradual decrease in PCV, Hb; lymphocytopenia and concomitant neutrophilia. Reduced serum proteins, lipids, cholesterol, triglycerides. Thickened gastric mucosa with mucin-filled cysts, moderately invasive gastric hyperplasia. Two-fold hepatomegaly, enlarged hepatocytes with increased SER; decreased liver DNA, RNA, increased MFO.	Allen, 1975
Rhesus monkey	F/ (8/group)	5 kg	Aroclor 1248/diet	0, 2.5 or 5.0 ppm for 11 months then treated with sheep red blood cells and tetanus toxoid.	After 6 months, monkeys developed chloracne, alopecia, and facial edema. Monkeys fed 5.0 ppm had significantly lower anti-sheep red blood cells antibody titer. Antibody response to tetanus toxoid was not measurably affected by PCB exposure. Both PCB fed groups had consistently lower gamma-globulin. Results indicate modest to slight immunosuppressive effects.	Thomas and Hinsdill, 1978

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
Rhesus monkey	NR/ (7)	NA	Aroclor 1248/ trans-placental or mother's milk trans-placental or mother's milk	Exp.1: mothers fed 2.5 ppm - compared w/ controls at 6 and 12 mos of age (concurrent exp. grp) Exp.2a: monkeys born from the same mothers after they had been off their 2.5 ppm diets from 0.5 to 1.5 yr. Exp. 2b: two grps monkeys from mothers fed 0.5 and 1.0 ppm 3 x/wk /3-4 mos.	Cumulative PCB intake averaged 293 mg. Significantly increased locomotor activity. Significantly retarded learning ability. In the 12-month tests, the mean data and variability of the two control groups were very similar, both in their mean locomotor activity levels in early sessions and in activity at about half that level in later sessions suggesting between session adaptation over the course of the experiment. In contrast, both the concurrent and the post-exposure 2.5 ppm groups showed between-session patterns which began at control activity levels but which rose to levels at least three times those of the controls by the final sessions. In the test conducted at 12 mos of age, locomotor activity for 15 min. periods within each daily session were within measurement error of being stable for all groups except the 0.5 and 1.0 ppm groups of exp. 2b. The former exhibited increasing activity throughout each session, a pattern which persisted across all 24 sessions run. On the other hand, the 1.0 ppm group showed within-session decrements which became less pronounced as the experiment continued. This latter pattern was the only one shown by any group which was consistent with the concept of "reactivity." Overall, all of the PCB treated groups were more active than their controls with no quantitative difference related to PCB dosage.	Bowman et al., 1981

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
CD-1 mouse	M/ (6/group)	19-24 g	Aroclor 1254 Emulphor: saline (1:8)/ oral	0, 10, 30, 100, 250 or 500 mg/kg bw. single dose	500 mg/kg; depression of spontaneous locomotor activity for <8 hours.	Rosin and Martin, 1983a
ICR mouse	M/ (5/group)	adult	Aroclor 1254/diet	0, 62.5, 250, 1000 or 4000 ppm for 14 days	4000 ppm: total mortality by day 7. 1000 ppm: death of 3/5 by day 15. 250 ppm: hepatomegaly, depressed food intake, decreased pentobarbital sleeping time. ≥62.5 ppm: elevated serum corticosterone. Weights of the testes, preputials, and vesicular glands were not affected.	Sanders et al., 1974.
CD-1 mouse	M/ (9/group)	25-29g	Aroclor 1254 Emulphor: saline (1:8)/oral	0, 30, or 100 mg/kg bw/for 14 days; 500 mg/kg single dose	500 mg/kg significantly enhanced pentobarbital sleeping time. 30 or 100 mg/kg for 14 days reduced pentobarbital sleep time.	Rosin and Martin, 1983b.
CL/ICR mouse	F/ (25/group)	sexually mature	Kanechlor 500/diet	0 or 500 ppm for 42 days	Depressed feed intake, hepatomegaly.	Tanimura et al., 1980.

*M=Male, F=Female, NR=not reported, NA= not applicable

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
DDY mouse	F/ (NR)	7-8 weeks	Kanechlor 500/95% ethanol/s.c.	0-10 mg/day for 10 days	Mortality at ≥4 mg/day.	Watanabe and Sugahara, 1981.
ICR mouse	NR/ (10)	4-wks	Kanechlor 500/diet	100, 200, or 400 µg for 21 days	Increased susceptibility to the lethal effects of infection with Herpes simplex or ectromelia virus. No significant difference in inducibility of interferon by polyinosinic acid-polycytidylic acid between PCB-fed mice and controls.	Imanishi et al., 1980
Swiss-Webster mouse	F/ (200)	6-8 weeks	Aroclor 1254/diet	10, 100 or 250 ppm for 12 wks. Animals were bred & exposed for 3 more weeks	No noticeable detrimental effect on reproduction and very little effect on the ability of pups to mount an immuno-response upon challenge with foreign antigens. Moderate liver cell hypertrophy at 100 and 250 ppm in the dams. No deleterious effects of PCBs on the growth of the dams.	Talcott and Koller, 1983
Balb/c mouse	M/5-6 wk-old/ (50/group)	NA	Aroclor 1254/diet	0 or 300 ppm/6 or 11 months	High mortality; 22 surviving mice fed 1254 for 11 months had greatly enlarged livers. Adenofibrosis was observed in the livers of all 22 mice but not in the groups fed for 6 months. Nine of the surviving mice fed 1254 for 11 months had hepatomas.	Kimbrough and Linder 1974

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Swiss Albino mouse	F/ (20/group)	adult	Group A: topical B[a]P and no 1254 in the diet; Group B: Aroclor 1254/diet and topical acetone; Group C: topical B[a]P and 1254 in the diet.	200 ppm Aroclor 1254 for 20 weeks? or until papillomas developed.	Various skin changes reported including hyperkeratinization in the epidermis, thicker pinnae, and erythema. However, the authors did not appropriately use a vehicle control for acetone.	Bell, 1983
DD mouse	M/ (114)	NA	Kanechlor 500/diet	0,100, 250 or 500 ppm for 32 weeks/32 weeks	Hepatomegaly in all treatment groups. Final body weight significantly decreased. Oval cell formation and bile duct proliferation at mid- and high dose levels of Kanechlor 500. Hepatocellular hypertrophy. Amyloidosis in all exposed groups except high-dose Kanechlor 500 groups: greater incidence associated with lower doses of lower chlorinated Kanechlors.	Ito et al., 1973a

*M=Male, F=Female, NR=not reported, NA= not applicable

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Balb/C mouse	M/ (25/group)	NA	Aroclor 1254/diet	0, 3.75, 37.5 or 375 ppm for 6 months/6 months	375 ppm 1254 and Moloney leukemia virus (MLV) had increased liver damage. All 1254 exposed groups: significant ($p<0.01$) increase in liver weight after 6 months. MLV increased the severity of hepatic lesions. Aroclor 1254: 375 ppm: significant mortality and severe hepatopathology; 3.75 ppm: no liver lesions.	Koller, 1977
Skh:HR-1 and HRS/ J mouse	F/ (3)	20-25g	Aroclor 1254, Phenoclor 54/acetone/ dermal	Aroclor 1254: 50 mg once; 1 mg 4 x wk for 6 wks; 8 mg, 4 x wk for 6 wks; Phenoclor 54 0.2 mg, 5 x wk for 10 wks.	50 mg Aroclor 1254 dose was fatal. Dosages of 1 and 3 (8?) mg induced no observable changes, either grossly or histologically. The skin of Phenoclor 54 treated mice appeared grossly normal, histology revealed hyperkeratosis and hyperplasia. Necropsy failed to show organ damage.	Puhvel et al., 1982
Fischer rat	M/ (10/group)	weanlings	Aroclor 1254/diet-pair feeding	0, 150, 300 ppm	Decreased rate of weight gain and food consumption; increased liver weight at all doses but no changes in kidney weights.	Carter and Mercer, 1983

*M=Male, F=Female, NR=not reported, NA= not applicable

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Sprague-Dawley rat	M/ (18)	250-300 g	Aroclor 1254/corn-oil/ oral	50 mg/kg for 7 days	Aroclor 1254 caused no significant chromosomal damage, and did not arrest the rate of spermatogenesis in the male rat. No alterations in the testes or epididymis were found, though an increase of acid-phosphatase activity of the interstitial tissue cells did occur.	Dikshith et al., 1975
Sprague-Dawley rat	M/ (4-6/group)	180-250 g 7 weeks	Aroclor 1254/mineral oil/oral	0, 50, 250, 500 mg/kg for 21 days	≥50 mg/kg bw/day: decreased rate of weight gain to frank weight loss; depressed feed intake; depressed water intake; depressed body temperature.	Komives, 1979; Komives and Alayoku, 1980
Wistar rat	F/ (40)	1 year	Aroclor 1254/corn-oil/oral	0, 12.5, 25, 50, 100, 400 800 mg/kg bw daily for 7 days	≥400 mg/kg: mortality; ≥100 mg/kg: increase liver fat percent ($p<0.05$) All doses: increased liver weight ($p<0.05$).	Grant and Phillips, 1974
Fischer rat	M/ (5/group)	34 days	Aroclor 1254/diet	0, 20 ppm for 1,2,4,8, or 14 days	Hepatomegaly by day four, 20 ppm represents a minimally toxic dose.	Carter, 1983

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Wistar rat	M, F/ (144)	30, 60 or 120 days	Aroclor 1254/corn-oil/oral	0, 5, 10, 20 mg/kg bw/day for 7 days	All doses: increased liver weights, increased aniline hydroxylase activity.	Grant and Phillips, 1974
Hooded rat	M/ (NR)	180-200 g	Aroclor 1254/diet	250 ppm for 14 days	Increased biliary excretion of T-4. Increased bile to plasma ratios of radiolabeled T-4. Increased biliary clearance of T-4. Decreased plasma concentration of radiolabeled T-4.	Bastomsky, 1974
Wistar rat	M, F/ (38)	NR	Aroclor 1254/diet	1000 ppm for 14 or 30 days	Moderate to severe vacuolar degeneration of periportal hepatocytes, hypertrophy, lipid accumulation and liver necrosis, decreased weight gain. Biosynthesis of cholesterol from [2-^{14}C]acetate and [2-^{14}C]mevalonate was decreased 51% and 31% respectively after 30 days, but no significant inhibition was observed after 14 days. Altered fatty acid synthesis.	Kling et al. 1978
NR/rat	M/ (NR)	NR	Aroclor 1254/NR/i.p.	100 mg/kg/day for 6 days	Aminolevulinic acid (ALA) synthetase activity increased, ALA dehydratase activity decreased, microsomal heme and cytochrome P-450 increased.	Watanabe and Sugahara, 1981.

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Osborne-Mendel rat	M/ (6/group)	8 weeks	Aroclor 1254/diet	0, 5, 50, 500 ppm for 4 weeks	≥5 ppm: enlarged thyroid, hypertrophy and hyperplasia of follicular cells and reduced follicular lumen, accumulation of large colloid droplets and irregular lysosomes, papillary projections and cytoplasmic projections, diluted RER.	Collins and Capen, 1980b
Gunn rat/ hetero- and homozy-gous	M/ (12/group)	300-400 g	Aroclor 1254/diet	500 ppm for 42 days	A 500 ppm dose caused ultrastructural alterations in the thyroid and a significant reduction in serum thyroxine in both heterozygous and homozygous rats. Also caused a reduction in serum triiodothyronine in heterozygous rats. The reduction in serum thyroid hormone levels was similar in both the hetero- and homozygous rat.	Collins and Capen, 1980a
Osborne-Mendel rat	M/ (5/group)	8 weeks	Aroclor 1254/diet	0, 50, 500 ppm for 4 or 12 weeks	A 50 and 500 ppm dose for 4 weeks caused an accumulation of lysosomal bodies and colloid droplets in follicular cells. Chronic administration also caused ultrastructural changes in follicular cells and a reduction in serum thyroxine. 35 weeks after cessation of feeding, thyroid follicular cells were similar to controls and serum thyroxine returned to normal.	Collins et al., 1977

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Sprague-Dawley rat	M/ (10/group)	100-130 g	Kanechlor 500/diet	100 ppm for 4 weeks	No decrease in food consumption. Only minimal effects on thymus, accessory sex organs, hematologic values, and liver weights were found. A significant increase in total serum protein and cholesterol, and a decrease in glucose and triglyceride concentration were reported.	Oishi et al., 1978
Holtzman rat	M/ (6-16/group)	250 g	Aroclor 1254/diet	0, 5, 50 or 500 ppm for 2, 3 or 5 weeks	Incidence of chromosome abnormalities and number of cells in mitosis from bone marrow or spermatogonial cells showed no consistent or dose-related change. 500 ppm:weight loss. ≥ 5 ppm: hepatomegaly. 500 ppm: slightly increased size of kidney, testes; decreased adipose. 50-500 ppm: blood-glucose reduced, BUN, cholesterol, protein increased. 5 ppm: only aminopyrine demethylase activity increased. 50-500 ppm: enzyme induction.	Garthoff et al.,1977
Holtzman rat	M/ (NR)	4 week-old	Aroclor 1254/diet	0, 5, 50, or 500 ppm for 5 weeks	≥5 ppm: enlarged thyroid, reduction in follicle size, hyperplastic cells with papillae and cytoplasmic processes extending into lumenal colloid; Follicular cells more columnar, mitochondria vacuolated with disrupted cristae, accumulation of colloid, increased number of lysosomes.	Kasza et al., 1978a

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Sprague Dawley rat	M/ (4/group)	100 g	Aroclor 1254 / diet	0 or 1000 ppm up to 6 weeks	Reduced rate of weight gain: Aroclor 1248>1254> 1260. Moderate elevation of Hb, PCV. Relative neutrophilia, lymphocytopenia. Enlarged liver, decreased thymus, fatty liver degeneration, cystic areas and focal necrosis with infiltration of inflammatory cells, ER proliferation, vesiculation of RER, increased number of lysosomes. Increased hepatic protein, RNA, phospholipid; decreased DNA, cholesterol. Induction of N-demethylase, nitroreductase.	Allen and Abraham-son, 1973
Wistar/ Neuher-berg rat	M/ (10/group)	100 g	Clophen A-50/olive-oil/ oral	0, 2, 10, 50, 150 or 250 mg/kg bw, twice/week for 6 weeks	≥2 mg/kg in rats produced an increase in serum cholesterol and triglycerides and histopathological alterations of the liver. ≥10 mg/kg doses caused an increase in liver weight and serum cholinesterase activity. ≥50 mg/kg dose caused an increase in serum bilirubin and protein. ≥150 mg/kg dose caused a significant increase in SGOT and SGPT.	Baumann et al., 1983
Long Evans rat	M/ (4-6/group)	3 weeks	Aroclor 1254 /corn-oil/i.p.	500 mg/kg single dose	Increased liver weight, decreased thymic weight; induction of various microsomal enzymes. Induction increased four-fold up to day 4.	Parkinson et al., 1983a

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal EffectsReference	
			Studies of 54% Chlorine PCB Mixtures			
Holtzman rat	M/ (NR)	4 week-old	Aroclor 1254/diet	0, 5, 50, or 500 ppm for 5 weeks	≥50 ppm: hepatomegaly, fatty degeneration, hepatocellular hypertrophy and cytoplasmic vacuolization. ≥5 ppm: increased liver weight, slightly enlarged SER decreased number of mitochondrial lysosomes; increased Golgi apparatus.≥50 ppm: Golgi apparatus decreased.	Kasza et al., 1978b
Wistar rat	M/ (NR)	young	Aroclor 1254/ Peanut-oil/i.p.	50 mg/kg/day for 3 days	Induction of the following hepatic enzymes: o-demethylase, aniline hydroxylase, carboxylesterase, bromosulfopthalein (BSP)-glutathione "conjugase," and p-nitrophenol-glucuronic acid "conjugase."	Ecobichon 1975
Sprague-Dawley rat	M,F/ (4/group)	6 week-old	Clophen A-50/olive oil/ oral	50 or 100 mg/kg bw 1 time/week for 7 weeks	Study examined the promoting effect of Clophen A50. 50 or 100 mg/kg Clophen A50 increased the number of ATPase-deficient islands 3-fold in males and 9-fold in females. Number and area of GGTase-positive islands were similarly enhanced. Increased liver weight.	Deml and Oesterle, 1982
Sprague-Dawley rat	M/ (24)	adult	Aroclor 1254/corn-oil/i.p.	1000 mg/kg	Did not alter the 24-hour periodicity in plasma corticosterone.	Dunn et al., 1983

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal EffectsReference	
			Studies of 54% Chlorine PCB Mixtures			
Sprague-Dawley rat	M/ (NR)	150-200 g	Aroclor 1254/Ringer's solution/i.p.	0, 25, 50 mg/kg once daily for 3 or 6 days	Increased liver weight at both doses. Six-fold increase in P-450. Increased NADPH cytochrome c reductase, ethylmorphine demethylase and inosine diphosphatase activity whereas glucose-6-phosphatase activity was decreased. Increased triglyceride to phospholipid ratio.	Hinton et al., 1978
Sprague-Dawley rat	M/ (NR)	100-120g	Aroclor 1254/NR/i.p.	100 mg/kg/day for 6 days or 100 mg/kg once a week for 6 weeks.	100 mg/kg for 6 days: elevated ALA synthetase, microsomal heme, cytochrome P-450. 100 mg/kg 1/wk for 6 weeks: no significant effect on ALA synthetase activity. ALA dehydratase and ferro cheletase activities were significantly decreased and there was a 2.5-fold increase in liver porphyrin content.	Alvares and Kappas, 1979
Sprague-Dawley rat	M/ (6)	5 wk-old	Kanechlor-400/olive-oil/oral	1 mg/rat/ day for 22 days	Increased SGOT and cholesterol levels, decreased triglycerides, IgG, leukocytes levels, and 11-hydroxycoritcosteroidserum levels. Induction of P-450.	Hori et al., 1986

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Table 3.2.5

. Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Wistar rat	M/ (120)	250g	Clophen A-50/corn-oil/i.p.	0 or 100 mg/kg bw, single dose up to 4 weeks	Cytochrome P-450 increased 3 to 4 fold, maximum in 1 week. NADPH activity doubled, P-nitroanisole-O-demethylase induced 6 to 7 fold, ~4-fold after 1 month. AHH activity increased 3-fold, down to normal ~1 month. Microsomal epoxide hydratase increased 2.5 fold at 1 week, persisted at least 4 weeks. Glutathione S-transferase increased at 1 day, remained at these levels. Microsomal UDP glucuronosyltransferase activity increased 2.5- fold in 1 week, persisted 4 weeks.	Parkki et al., 1977
Wistar rat	M/ (NR)	250-300 g	Aroclor 1254 mixture/corn-oil/oral	500 or 1000 mg/kg single dose	Reduced norepinephrine concentration in frontal cortex, hippocampus but not in the hypothalamus and brainstem. These effects were reversible and dependent upon the presence of PCBs.	Seegal et al., 1985a
Wistar rat	M/ (9/group)	250-300 g	Aroclor 1254 mixture/corn-oil/oral	500 or 1000 mg/kg single dose	Decreased dopamine (DA) and 3,4-di-hydroxyphenylacetic acid (DOPAC) in caudate. DA concentrations in olfactory tract unaffected. Result show that brief exposures to PCBs can cause regional differences in brain neurochemistry.	Seegal et al., 1986

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Rats, dogs chickens	NR	NR	Aroclor 1254/diet	1, 10, and 100 ppm rats and dogs 18 mos. Duration of rat and chicken repro-duction study not reported.	After 18 months rats fed Aroclor 1254 had increased liver weights at 100 ppm but not at 1 or 10 ppm. No other effects were observed. Dogs fed Aroclor 1254 did not gain weight as well as controls. Decreased survival of pups was found at 100 ppm Aroclor 1254. In chickens, weight loss, decreased eggshell thickness and poor hatchability was found at 100 ppm of 1254. At at other levels of 1254 there were no effects.	Keplinger et al., 1971 (abstract)
Wistar rat	M/ (6/group)	110 g	Kanechlor 500/diet	500 ppm for 50 days	Decreased weight gain and increased liver weight. Decreased thiamine levels in blood, liver, and sciatic nerve. Decreased transketolase activity in erythrocytes and liver.	Yagi et al., 1979
Osborne-Mendel rat	M/ (6)	100-200g	Aroclor 1254/diet	0.05, 0.5 and 4.7 mg/kg for 30 days	4.7 mg dose caused increased liver weight and induced cytochrome P-450. 0.5 mg/kg dose did not increase liver weight, protein content or induce cytochrome P-450. The 0.05 mg/kg dose also did not induce P-450.	Litterst and Van Loon, 1972

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Wistar rat and White Russian rabbit	M/ (NR)	rabbits 1400-1800 g; rats 100-200 g.	Clophen A-50/ olive-oil/diet	100 ppm daily for 5 days.	Rabbits: liver weights and cytochrome P-450 increased whereas microsomal levels of aminopyrine and p-nitroanisole-demethylation, aniline and 4-chlorobiphenyl-p-hydroxylation were unchanged. In rat liver microsomes, P-450 levels were increased 2-5 fold. Hexobarbital half-life in rabbits remained unchanged when hexobarbital sleeping time was reduced by more than 50% after the same dose in rats.	Wolff and Hesse, 1977
Sprague-Dawley rat	M/ (25/group)	NA	Aroclor 1254/ diet	0 or 100 ppm for 52 weeks/ 65 weeks	Liver homogenates had an increased hepatic protein, RNA and lipid content; decreased DNA. Increased microsomal total protein and cytochrome P-450. Induced N-demethylase, nitroreductase. Inhibited glucose 6-phosphatase.	Allen and Abraham-son,1979
Sherman rat	M, F/ (10/group)	NA	Aroclor 1254/diet	0, 20, 100, 500, ppm for 8 months/ 8 months	Mortality (3/20) and reduce rate of weight gain at 500 ppm. Hepatomegaly, enlarged hepatocytes with foamy cytoplasm-containing inclusions at ≥20 ppm. Adenofibrosis, fat, and pigment accumulation at ≥100 ppm. The effect of Aroclor 1254 was greater than that of 1260.	Kimbrough et al., 1972

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Sprague-Dawley rat	M/ (10/group)	100 g	Aroclor 1254	0, 1, 5, and 25 ppm for 35, 70 or 140 days and 30 mg/kg pento-barbital, i.v.	After 35 days of pre-treatment only the 25 ppm treated rats showed significant acceleration of pentobarbital elimination. After 70 and 140 days pretreatment, the 5 and 25 ppm treated rats showed significant acceleration of pentobarbital elimination. The 1 ppm pretreatment had no effect on pentobarbital pharmacokinetics.	Chu et al., 1977
F-344 rat	M,F/ (191)	NA	Aroclor 1254/diet	0, 25, 50, 100 ppm/2 years	Reduced body weight. Stomach: dose-related metaplasia of stomach; adenocarcinoma of glandular stomach.	Morgan et al.,1981
Wistar rat	M/ (290)	NA	Kanechlor 500/diet	0, 100, 500 or 1000 ppm for 28-52 weeks/ 1 year	Heavy mortality. Hepatomegaly, oval cell and bile duct proliferation, fatty liver infiltration. Cholangiofibrosis at 1000 ppm level, nodular hyperplasia. Depressed final body weight. No hepatocellular carcinoma. No remarkable changes were seen in other organs.	Ito et al., 1974
Sherman rat	M/ (50/group)	NA	Aroclor 1254/diet	500 ppm for 6 months/10 months	Increased liver weight, adenofibrosis, lipid accumulation. 10 mos. after exposure had ceased 1192 ppm PCBs were present in adipose and 22.65 ppm in the liver.	Kimbrough et al., 1973

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
CD rat	F/ (300)	NA	Aroclor 1254/diet	0, 10, 30 or 100 ppm for up to 20 weeks/20 weeks	Serum cholesterol, beta globulin increased, gamma globulin decreased (dose-related). ≥30 ppm: reduced rate of weight gain, hepatomegaly, cardiomegaly (dose-related). ≥10 ppm: hepatic porphyrinic fluorescence. ≥10 ppm: erythema, crustiness, hyperkeratosis, perikeratosis on ears, dorsum of nose and feet, tail.	Zinkl, 1977
Sherman rat	NR	NA	Aroclor 1254/diet	500 ppm/6 months	Pancreatic-type tissue induced in the livers of rats. The tissue was found to be identical morphologically and functionally to pancreatic acinar tissue.	Rao et al.,1986
Sprague-Dawley rat	M/ (96)	NA	Aroclor 1254/diet	0 or 100 ppm for 52 weeks/65 weeks	Normal appetites, appearance, weight gain, Hb, PCV, WBC, serum protein, A/G ratios. Elevated serum total lipids, cholesterol. Total lipid and triglyceride spiked very high peaks on Aroclor 1254 (only) at 52 weeks. Cholesterol levels persisted at 65 weeks (13 weeks off exposure). Triglyceride levels fell less than controls by 65 weeks. Hepatomegaly: focal degeneration and necrosis by 13 weeks.	Allen et al., 1976
Sprague-Dawley rat	M/ (6)	weanling	Aroclor 1254/diet	50 or 500 ppm for 10 wks	Significantly suppressed splenic natural killer (NK) cell activity.	Talcott et al., 1985

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs /Vehicle/Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
New Zealand rabbit	M, F/ (20/group)	NR	Aroclor 1254/ corn-oil/oral	0 or 300 mg 1 time weekly for 14 weeks	Aroclor 1254: elevated SGOT, SGPT. Aroclor 1254: slight transient increase in serum cholesterol, reduced rate of weight gain, severe hepatomegaly, uterine atrophy. No differences in hematologic parameters, BUN, or serum protein fractions. Histopathologic changes, Aroclor 1254: vacuolated and granular enlarged hepatocytes, centrilobular necrosis and fibrosis. Ballooned RER.	Koller and Zinkl, 1973.
New-Zealand rabbit	M/ (7/group)	~2kg	Aroclor 1254/diet	0, 3.7, 20, 45.8, 170 ppm diet for 8 weeks: 0.18, 0.92, 2.1 or 6.54 mg/kg bw/day, respectively	No effect on feed consumption, growth rate, visceral pathology except hepatomegaly which was statistically significant (p=0.05) at the highest two doses. Only slight liver enlargement. No effect on hematologic parameters. No consistent immunological response (hemolysis or hemagglutination titers) to sheep RBC. Gamma globulin reduced at all levels. Dose related decreases in splenic and thymic gamma globulin producing cells.	Street and Sharma, 1975

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 54% Chlorine PCB Mixtures			
Yorkshire pig	NR/ (2/group)	14 days	Aroclor 1254/ olive-oil, dispersed by Tween 60 in condensed milk then added to diet	0, 12.5, 25, 50, 100 mg/kg bw up to 35 days	Deaths occurred in 11 to 35 days. All levels: partial anorexia, reduced weight gain, diarrhea abdominal distension, gastritis, colitis, enlarged thyroid,thymic and splenic atrophy, liver and kidney enlargement. Germ-free pigs appear to be more sensitive to the acute effects of PCBs.	Miniats et al., 1978
Japanese quail	M/ (5-8)	Adult	Aroclor 1254/corn-oil/ i.p.	100 mg/kg, single dose	Increased hepatic weight and benzopyrene hydroxylase activity which was continuous up to 168 h after the treatment. At 96 h, diamine oxidase activity was decreased.	Ignesti et al., 1986
Mink	F/ (7-8/group)	NA	Aroclor 1254/diet	0 or 2 ppm/10 months	Aroclor 1254: interference with reproduction. All Aroclors tested: no significant differences in body weight gain, hemoglobin, PCV.	Aulerich and Ringer, 1977
Cynomolgus monkey	F/ (10)	2.5 kg	P-KC-400/olive-oil/oral	5 mg/kg daily/20 wk	Gradual weight loss, reduced serum cholesterol levels, decreased humoral immune response, liver induction and hypertrophy.	Hori et al. 1982

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal EffectsReference	
			Studies of 54% Chlorine PCB Mixtures			
Cyno-molgus monkey	F/ (4)	NA	Aroclor 1254/corn-oil, gelatin, apple juice	0, 100 or 400 µg/kg bw/day, 3 days/wk/up to 238 days	Two monkeys receiving 100 µg/kg/day delivered stillborn infants and the 400 µg/kg/day monkey delivered a term infant that had impaired immunologic function, and died at 139 days post-partum.	Truelove et al., 1982
			Studies of 60% Chlorine PCB Mixtures			
NMRI mouse	F/ (45)	sexually mature	Clophen A-60/peanut-oil/oral	0 or 0.025 mg/mouse daily for 10 weeks	Increased length of estrus cycle and the frequency of implanted ova was lower than controls.	Orberg and Kihlstrom, 1973
Sprague-Dawley rat	M, F/ (24/group)	300g	Phenoclor DP6/diet	0, 100 ppm for 8 days	Decreased phenobarbital sleeping time, increased liver weight and protein content; males were affected to a greater extent than females.	Narbonne, 1979
Sprague-Dawley rat	M,F/ (6/group)	90 g young rats; 300-350 g old rats	Phenoclor DP6/diet	100 ppm for 8 days; 13 mg/kg adults; 24 mg/kg young for 8 days	100 ppm males: phenobarbital sleeping times reduced, liver weight and total protein content increased. 100 ppm females: small increase in liver weight and protein content. 13 and 24 mg/kg dose levels, liver weight elevated equally in both sexes; elevated liver protein and fat content was higher in adults	Narbonne, 1979

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Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 60% Chlorine PCB Mixtures			
Sprague Dawley rat	M/ (4/group)	100 g	Aroclor 1262/ diet	0 or 1000 ppm up to 6 weeks.	Reduced rate of weight gain: Aroclor 1248>1254> 1260. Moderate elevation of Hb, PCV. Relative neutrophilia, lymphocytopenia. Enlarged liver, decreased thymus, fatty liver degenerations, cystic areas and focal necrosis with infiltration of inflammatory cells ER proliferation, vesiculation of RER, increased number of lysosomes. Increased hepatic protein, RNA, phospholipid; decreased DNA, cholesterol. Induction of N-demethylase, nitroreductase.	Allen and Abrahamson, 1973
Wistar rat	M (NR)	250-300 g	Aroclor 1260 mixture/corn-oil/oral	500 or 1000 mg/kg single dose	Reduced norepinephrine concentration in frontal cortex, hippocampus but not in the hypothalamus and brainstem. These effects were reversible and dependent upon the presence of PCBs.	Seegal et al., 1985a
Wistar rat	M / (9/group)	250-300 g	Aroclor 1260 mixture/corn-oil/oral	500 or 1000 mg/kg single dose	Decreased dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in caudate. DA concentrations in olfactory tract unaffected. Results show that brief exposures to PCBs can cause regional differences in brain neurochemistry.	Seegal et al., 1986

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Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 60% Chlorine PCB Mixtures			
Wistar rat	M/ (NR)	young	Aroclor 1260/ Peanut-oil/i.p.	50 mg/kg/day for 3 days	Induction of the following hepatic enzymes: o-demethylase, aniline hydroxylase, carboxylesterase, bromosulfopthalein (BSP)-glutathione "conjugase," and p-nitrophenol-glucuronic acid "conjugase."	Ecobichon 1975
Rats, dogs chickens	NR	NR	Aroclor 1260/diet	1, 10, and 100 ppm rats and dogs 18 mos. Rat repro-duction study duration not reported. Chicken repro-duction study duration not reported..	100 ppm Aroclor 1260 treated animals did not gain weight as well as controls. No adverse effects at any of the 3 levels of 1260. In chickens, 100 ppm 1260 had no effect on hatchability or eggshell thickness.	Keplinger et al., 1971 (abstract)

*M=Male, F=Female, NR=not

NA= not applicable

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 60% Chlorine PCB Mixtures			
Sherman rat	M, F/ (10/group)	NA	Aroclor 1260/diet	0, 20, 100, 500, 1000 ppm for 8 months/8 months	Mortality 1/10, 2/10, 8/10 of females in 100, 500, 1000 ppm groups. Decreased rate of gain at ≥500 ppm Hepatomegaly, male and female at ≥20 ppm, discolored livers with UV fluorescence, enlarged hepatocytes with foamy cytoplasm-containing inclusions. Increased lipid content at ≥100 ppm. Pigment accumulation at 500 ppm. Adenofibrosis at ≥100 ppm.	Kimbrough et al., 1972
Wistar rats	F/ (8/group)	NR	Phenoclor DP6/diet Clophen A60 and Aroclor 1260	2000 ppm	100% mortality in 12-56 days; hepatomegaly, centrilobular necrosis.	Vos and Koeman, 1970
Sprague-Dawley rat	M/ (25/group)	NA	Aroclor 1262/diet	0 or 100 ppm for 52 weeks/65 weeks	Liver homogenates had an increased hepatic protein, RNA and lipid content; decreased DNA. Increased microsomal total protein and cytochrome P-450. Induced N-demethylase, nitroreductase. Inhibited glucose 6-phosphatase.	Allen and Abraham-son,1979

*M=Male, F=Female, NR=not reported, NA= not applicable

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 60% Chlorine PCB Mixtures			
Guinea pig	M/ (40)	3-4 weeks/ -225 g	Clophen A-60/diet	0, 10, 20, 250 ppm for 4 weeks	250 ppm: 80% mortality; cachexia, lymphoid atrophy, liver damage, hepatomegaly ≥50 ppm. ≥10 ppm: dose-related reduction in hemagglutination titers and tetanus antitoxin-producing cells in popliteal lymph nodes induced by tetanus toxoid injection.	Vos and Van-Driel-Grooten-huis, 1972
Guinea pig	F/ (40)	3-4 weeks/ -225g	Aroclor 1260/diet	50 ppm for 6 weeks	Significant reduction of tetanus anti-toxin titers, circulating leukocytes and lymphocytes and thymus atrophy with both PCBs at 50 ppm. 100% mortality at 250 ppm. Reduced tuberculin skin reaction, thymus atrophy, leukopenia at 50 ppm.	Vos and van Genderen, 1973
	F/(40)		Clophen A-60/diet	0, 10, 50 ppm for 8 weeks		
Guinea pig	F/ (3[illegible])	~220g/ 4 weeks	Aroclor 1260/diet	0, 10, 50 ppm for 8 weeks	50 ppm: reduced weight gain, hepatomegaly, reduced weight gain of kidney, adrenals. 10 ppm: splenic atrophy, reduced popliteal lymph node, gamma-globulin-producing cells, decreased serum gamma-globulin concentration in animals given tetanus toxoid injection.	Vos and de Roij,1972
New Zealand rabbit	F/ (40)	3-4 weeks/ -225g	Clophen A-60/diet	0, 10, 50 ppm for 6 weeks	50 mg/kg Clophen: increased liver weight.	Vos and Van-Driel-Grooten-huis, 1972

*M=Male, F=Female, NR=not reported, NA= not applicable

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 60% Chlorine PCB Mixtures			
New Zealand rabbit	F/ (16)	2500-3050 g/ 5 months	Phenoclor DP6 Clophen A-60 Aroclor 1260/ isopropanol/ dermal	118 mg/day, 5 days/ week for 38 days (27 applications)	All PCBs: Gradual weight loss, mortality beginning at day 10, erythema and thickening of skin, subcutaneous edema, ascites, UV fluorescence. Fecal coproporphyrin and protoporphyrin elevated. Hematology: leukopenia. Apparent hepatomegaly, kidney enlargement. Liver: centrilobular degeneration, focal hydropic degeneration, focal necrosis, centrilobular hepatocytic atrophy (more pronounced in Clophen, least in Aroclor group), periportal fibrosis. Kidney: hydropic degeneration of convoluted tubules, pyknotic nuclei, rhexis and lysis of tubular epithelial cells, tubular dilation with casts of necrotic cells.	Vos and Beems, 1971
New Zealand rabbit	F/ (12)	2.5-2.9 kg 3.5 months	2,4,5,2',3',5'-hexa-CB, Aroclor 1260/ isopropanol/ dermal	120 mg/day, 5 days/ week, for 4 weeks (20 applications)	Dermal: erythema, wrinkling, hyperkeratosis, reduced hair regrowth; more severe in Aroclor 1260 group. Hepatomegaly, elevated SGPT, SGOT in 2,4,5-hexa-CB group. Dermal sections: epidermal follicular hyperplasia; follicular plugging more in Aroclor 1260 group. Moderate thymic atrophy in both PCB groups. Liver lesions coproporphyrin elevated in both similar to previous study (Vos and Beems, 1971), more severe in 2,4,5-hexa-CB groups.	Vos and Notenboom-Ram, 1972

*M=Male, F=Female, NR=not reported, NA= not applicable

Table 3.2.5

Acute, Subchronic, and Chronic Toxicities of Commercial PCB Mixtures

Strain/ Species	Sex/No.*	Weight/ Age	Source of PCBs/Vehicle/ Route	Dose/ Duration	Animal Effects	Reference
			Studies of 60% Chlorine PCB Mixtures			
NR/ chickens	F/ (20-24/group)	1-day-old	Phenoclor DP6/diet Clophen A60 and Aroclor 1260	400 ppm up to 60 days All chicks fed Clophen A-60 or Phenoclor DP6 died during the experiment	Microscopically centrilobular necrosis in animals fed clophen A-60 and Phenoclor DP6 whereas atrophy of the spleen was found in all groups. Chemical porphyria was found as a general PCB effect.	Vos and Koeman, 1970
Japanese quail	F/ (5/group)	112-130 g	Phenoclor DP6/diet Clophen A60 and Aroclor 1260	2000 ppm duration unknown	100% mortality in 5-13 days.	Vos and Koeman, 1970

*M=Male, F=Female, NR=not reported, NA= not applicable

3.3 MECHANISMS OF TOXICITY

Despite the considerable study of the toxicity of the PCBs, relatively little is known about the manner in which toxic effects are produced. Two fundamental mechanisms have been proposed. One potential mechanism involves the formation of chemically-reactive metabolites of the PCBs which bind to critical cellular macromolecules to produce toxicity. The second proposed mechanism involves the combination of the PCB with a specific cytosolic receptor which initiates a sequence of biochemical events leading to toxicity.

3.3.1 Formation of Reactive Metabolites

The theory of the reactive metabolite mechanism originates from the observation that many PCB congeners are hydroxylated through the formation of an arene oxide. This is a normal pathway of metabolism, and the formation of an hydroxylated product is important in the elimination of PCBs (see Section 3.2, Pharmacokinetics of PCBs). Since epoxide metabolites are usually reactive and can be quite toxic, it has been proposed that arene oxide intermediates of PCBs might lead to the alkylation of important cellular macromolecules. In support of this theory, Shimada (1976) observed that PCB mixtures became covalently bound to hepatocellular proteins *in vivo* in a process that required metabolism. Shimada and Sato (1978) subsequently demonstrated *in vitro* the metabolic activation of PCBs by cytochrome P-450, and that the resulting binding could be inhibited by glutathione and cysteine. Results from this study and another report (Shimada and Sato, 1980) suggested that phenobarbital-inducible forms of cytochrome P-450 were responsible for the bioactivation of the PCBs in rat, mouse, and rabbit liver microsomes. These investigators made similar observations in a later report when PCB-binding activity was studied in purified cytochrome P-450 from phenobarbital- or 3-methylcholanthrene-treated rabbits (Shimada et al., 1981).

The formation of covalently-binding PCB metabolites does not appear to be exclusively a property of phenobarbital-inducible forms of cytochrome P-450, however. The ability of cytochrome P-450 fractions from

3-methylcholanthrene-treated animals to produce covalent binding during the metabolism of labelled Kanechlor 300 suggests that 3-methylcholanthrene-inducible forms may produce reactive metabolites from at least some PCB congeners. When Shimada and Sawabe (1983) examined the covalent binding of 2,4,2',4'-tetrachlorobiphenyl and 3,4,3',4'-tetrachlorobiphenyl in hepatic microsomal suspensions from phenobarbital- and 3-methylcholanthrene-treated rats, it was found that binding of the 3,4,3',4'-tetrachlorobiphenyl occurred most actively in microsomes from the rats treated with 3-methylcholanthrene.

Other investigators have also observed the formation of covalently-binding products from the metabolism of PCBs. Seymour et al. (1976) found that the microsomal metabolism of 2,5,2',5'-tetrachlorobiphenyl in rats resulted in t binding of radiolabel to proteins that could not be removed by hot methanol extraction. Morales and Matthews (1978) found a significant irreversible binding of radiolabel *in vivo* after administration of 2,3,6,2',3',6'-hexachlorobiphenyl to mice.

Targets for binding of reactive PCB metabolites potentially included proteins, DNA, RNA, and other critical macromolecules. Binding to proteins appears to be non-random. Hargraves and Allen (1979) found that phenobarbital induction of rats not only substantially increased the *in vitro* hepatic microsomal protein binding resulting from metabolism of 2,2',5,5'-tetrachlorobiphenyl, but also that the binding appeared to be associated with a protein of about 45,000 daltons. The nature of this protein was not determined. Shimada and Sawabe (1983) found that the metabolite(s) of 3,4,3',4'-tetrachlorobiphenyl bound non-randomly to cytochrome P-448 and to two cytosolic proteins, approx. 26,000 and 46,000 daltons. These cytosolic proteins were not characterized. The investigators did, however, evaluate a number of proteins as targets for metabolite binding. Only those with a free sulfhydryl group showed significant binding, indicating that the tetrachlorobiphenyl metabolite(s) preferentially binds to sulfhydryls.

Narbonne and Daubeze (1980) found that *in vitro* metabolism of 2,4,5,2',4',5'-hexachlorobiphenyl resulted not only in binding to protein, but also in

binding to added calf thymus DNA. Interestingly, when hepatic microsomal suspensions from PCB-induced (Phenoclor DP6, a French commercial PCB mixture) rats were used, binding to both protein and DNA were minimal. The authors proposed that the apparent loss of binding in the incubations with PCB-induced microsomes was due to competition between *in vivo* and *in vitro* added PCB for saturated binding sites. While PCB-pretreatment of the liver-donor rats could conceivably result in a loss of sites available for subsequent reactive metabolite binding *in vitro*, there is no explanation for why sites on the DNA added during the *in vitro* incubations should be altered. Another, perhaps more plausible explanation for their results is that PCB-pretreatment results in the loss of the form(s) of cytochrome P-450 responsible for production of the reactive metabolite. If this were true, then reactive metabolite formation would be self-limiting in a few days and could not possibly account for the toxic effects of PCBs which typically develop over a longer period of time. Though binding to DNA was demonstrated, it is doubtful that this is of toxicological significance because repeated studies have found that PCBs are not genotoxic (see Section 3.6, Genotoxic Effects of PCBs).

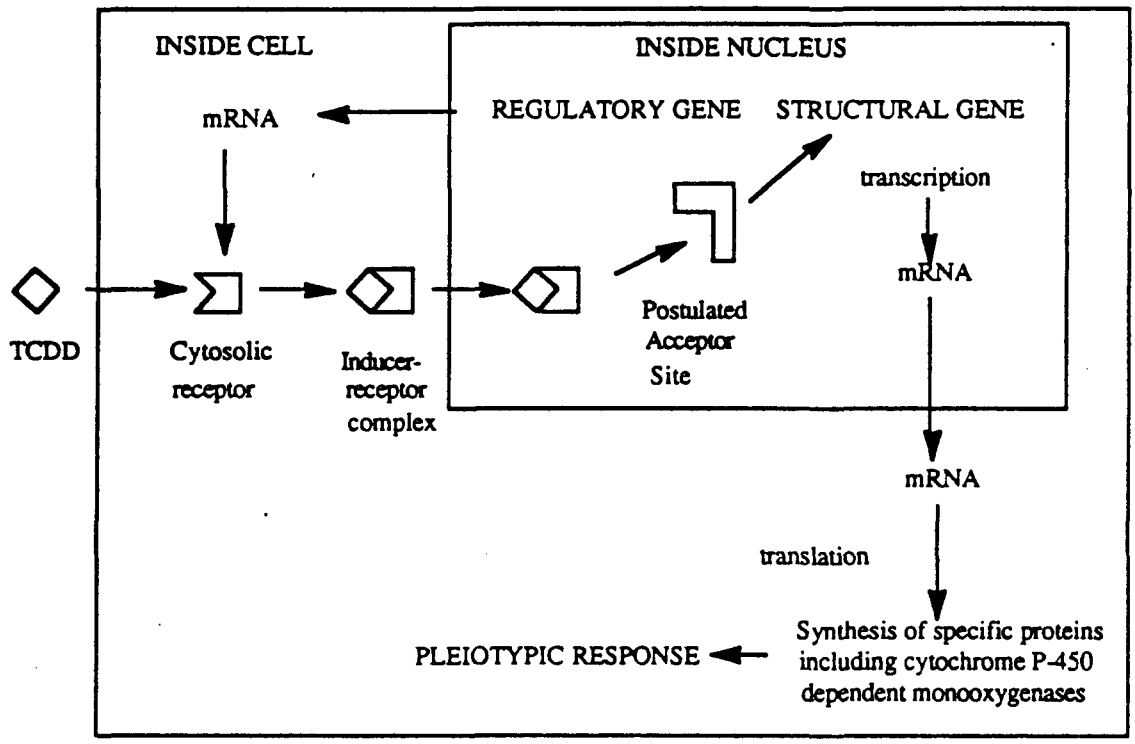
Shimada has recently published a study which indicates that a dosing regimen of 3,3',4,4'-tetrachlorobiphenyl causing thymic atrophy produces no detectable covalent binding of PCB in thymic tissue (Shimada, 1987). The author concludes that reactive metabolite formation does not contribute to thymic atrophy from PCBs, apparently dismissing the concept that reactive metabolites may cause thymic toxicity through indirect mechanisms. Even in the absence of negative studies such as this one, however, the reactive metabolite mechanism has not received widespread support. Perhaps the most important weakness in this theory is the general observation that PCB congeners most likely to undergo arene oxide formation are the congeners that are the least persistent. These are of course related events, as metabolism appears to be a prerequisite for elimination (see Section 3.1, Pharmacokinetics). The least persistent PCB congeners are also generally the least toxic, so there is an overall inverse relationship between susceptibility for arene oxide formation (and therefore reactive metabolite production) and toxic potential.

3.3.2 Toxicity Mediated by *Ah* Receptor Binding

A number of different types of halogenated aromatic hydrocarbons (HAHs) have been found to produce similar toxic effects in animals. These characteristic effects in animals include lethality (usually preceded by a wasting syndrome), skin disorders, hepatotoxicity, immunotoxicity, porphyria, thymic involution, and an edema syndrome. The appearance of these toxic symptoms may be species dependent, and potency in producing these effects can vary widely, even among specific congeners of a particular group of HAHs such as PCBs. However, the qualitative similarity of toxic symptoms shared by this broad class of organic compounds suggests that these effects may be produced by a common mechanism. Many of the studies of this potential mechanism of toxicity involve HAHs other than the PCBs. Since it is proposed that HAHs work through the same toxic mechanism, studies of other HAH compounds, especially TCDD and PCDFs, are relevant to a discussion of the mechanism of PCB toxicity.

Another characteristic effect of the HAHs is the induction of cytochrome P-448, a name given to the cytochrome P-450 forms characteristically induced by polycyclic aromatic hydrocarbons such as 3-methylcholanthrene. Detailed studies have indicated that this induction is initiated by the combination of the inducing agent with a specific cytosolic receptor, termed the *Ah* receptor. A discussion of the mechanism as it relates to enzyme induction appears in the Section 3.2.3, Effects on the Liver. It has been proposed that many of the toxic effects of the HAHs, including the PCBs, also are initiated by the combination of the HAH with the *Ah* receptor. Binding to the *Ah* receptor is thought to lead to a pleiotrophic response. Cytochrome P-448 induction is but one manifestation of this response, and toxicity may result from this induction or other aspects of the pleiotrophic response through mechanisms which have yet to be identified (Poland et al., 1979; Safe, 1984; Figure 3.3.1).

Much of the evidence in support of this theory has come from comparative studies of HAH toxicity in so-called "responsive" and "non-responsive" inbred



Schematic of *Ah* Receptor Binding

Figure 3.3.1

mouse strains. Some inbred strains of mice will respond with enzyme induction when dosed with 3-methylcholanthrene while other inbred strains will not. Response is typically measured by induction of aryl hydrocarbon hydroxylase (AHH) activity. The prototype "responsive" mouse is the C57BL/6J and the prototype "non-responsive" mouse is the DBA/2J. Experiments involving genetic crosses and backcrosses of C57BL/6J and DBA/2J mice have shown that the ability to respond to 3-methylcholanthrene with an increase in AHH activity was found to be inherited in a simple autosomal dominant mode, controlled by the *Ah* locus. (Nebert and Gielen, 1972; Nebert et al., 1972; Thomas et al., 1972).

When HAHs in general are considered, the differences between the "responsive" and "non-responsive" strains with respect to AHH induction appears to be related to potency rather than absolute differences. Tetrachlorodibenzodioxin (TCDD), for example, produces induction of AHH activity in the "non-responsive" DBA/2J mice, but the induction requires approximately 10-fold higher doses than those required for induction in C57BL/6J mice (Poland et al., 1974; Poland et al., 1979; Poland and Glover, 1980). Differences between the responsive and non-responsive strains appear to be a function of the amount or quality of the *Ah* receptor protein, and binding studies have confirmed that the *Ah* receptor found in the responsive C57BL/6J mouse is absent or undetectable in the DBA/2J mouse (Hannah et al., 1981; Gasiewicz and Rucci, 1984).

Since the basis for responsiveness to AHH induction from HAHs appears to relate to *Ah* receptor differences, if toxicity were also mediated through this receptor there should be substantial differences in susceptibility to HAH toxicity between responsive and non-responsive strains. In general this appears to be the case. When thymic involution from TCDD exposure was used as the endpoint, Poland and Glover (1980) found approximately an order of magnitude difference in potency in TCDD between C57BL/6J and DBA/2J mice. Sensitivity appeared to segregate with the *Ah* locus in cross-breeding and backcross experiments. These strains also appear to have different sensitivities to teratogenic effects of TCDD in mice (Courtney and Moore, 1971), and this sensitivity also segregates with the *Ah* locus (Poland and Glover, 1980). Similar observations of differences in sensitivity to

toxicity between responsive and non-responsive strains have been reported for hepatic porphyria due to a decline in uroporphyrinogen decarboxylase activity (Jones and Sweeney, 1977; Jones and Sweeney, 1980).

Differential sensitivity to the effects of HAHs is not confined to TCDD, and Silkworth and Grabstein (1982) have studied PCB immunotoxicity in C57BL/6J and DBA/2J mice. Mice from both strains were administered either the planar congener 3,4,3',4'-tetrachlorobiphenyl or the nonplanar congener 2,5,2',5'-tetrachlorobiphenyl in dosages of 0, 10, or 100 mg/kg ip two days before and two days after immunization with sheep red blood cells (SRBC). The coplanar 3,4,3',4'- congener binds to the *Ah* receptor with relatively high affinity while the noncoplanar 2,5,2',5'-tetrachlorobiphenyl does not. Immunotoxicity was evaluated by determining the number of plaque-forming cells in the spleen five days after a second immunization. Only the 3,4,3',4'-tetrachlorobiphenyl congener was immunotoxic, producing up to 90% loss of anti-SRBC antibody-forming cells per spleen, and only in the C57BL/6J mouse. 3,4,3',4'-Tetrachlorobiphenyl was not immunotoxic in the DBA/2J mouse, and 2,5,2',5'-tetrachlorobiphenyl was not toxic in either strain. Parkinson et al. (1982) have used a different toxic endpoint, thymic atrophy, to determine the sensitivity of C57BL/6J and DBA/2J mice to PCB toxicity. The effect of 2,3,4,4',5-pentachlorobiphenyl administration on thymic weight was measured in both strains. This PCB congener produced a dose-dependent decline in thymus weight in the "responsive" strain (C57BL/6J) but not the "non-responsive" strain (DBA/2J).

The second line of evidence for a role for cytosolic *Ah* receptor binding in HAH toxicity comes from studies showing a correlation between structure-activity relationships for AHH induction (by virtue of *Ah* receptor binding) and structure-activity relationships for toxicity. Several studies have compared the structural requirements for AHH induction with the structural requirements for toxicity. In general, those HAH congeners which induce AHH are the most toxic while those that produce a phenobarbital-like induction are relatively non-toxic (Yoshimura et al., 1979; Safe et al., 1982; Safe, 1986; Poland and Knutson, 1982; Safe et al., 1985; Leece et al., 1985; Brunstrom and Andersson, 1988). The

structural attributes of HAH molecules which facilitate *Ah* receptor binding therefore appear to be associated with toxicity.

An important confounder to consider in attempting to use these correlations to ascribe PCB toxicity to *Ah* receptor binding is the fact that the congeners which are most toxic are also the congeners which tend to be most persistent. For maximal AHH induction and molecular coplanarity, the chlorines must be located at the *meta-* and *para-* positions of the biphenyl nucleus. As discussed in Section 3.2, Pharmacokinetics of PCBs, this pattern of chlorination decreases metabolism because the *para-* position is hindered and the favored vicinal carbons for oxidative attack are likewise halogenated. This means that congeners proposed as toxic because they are AHH inducers (and bind the *Ah* receptor) should be obviously more toxic on the basis of their bioaccumulation potential alone when compared to phenobarbital-type inducers which are more readily metabolized and excreted. While this fact is relatively obvious, little has been done to demonstrate that the differences in toxicity are not merely differences in the ease of metabolism, i.e. bioaccumulation potential, that are known to exist between isomers.

Gasiewicz et al. (1983) have presented data which indicate that not only congener differences in disposition, but also strain differences in disposition of HAHs may contribute to apparent differences in toxic potency. These investigators administered equivalent doses of TCDD to C57BL/6J and DBA/2J mice, and to the cross strain, B6D2F1. It was found that the DBA/2J mouse had a significantly larger percentage of body weight as fat, and was able to sequester a larger percentage of the TCDD dose in adipose tissue stores. Consequently, TCDD concentrations in some organs including the liver were much smaller in the DBA/2J mouse, perhaps accounting for at least some of the sensitivity differences between this "non-responsive" strain and the C57BL/6J strain.

One potential approach to structure-activity relationship comparisons which would minimize the effects of variable disposition is the use of cell cultures. There is very little information regarding PCB congener structure-activity relationships for AHH induction and toxicity in cell cultures. Cell culture experiments for other

HAHs, however, have not provided data which support a common mechanism for AHH induction and cellular toxicity. Knutson and Poland (1980a) examined twenty-three different cell culture lines for induction of AHH activity and toxicity by the addition of TCDD. Nine of the cell culture lines had basal levels of AHH activity, and eight of the nine were induced by TCDD exposure in culture. Despite this evidence of *Ah* receptor interaction, no toxicity was observed in any of the cultured cell lines. Others have also exposed cell culture lines to TCDD and observed AHH induction without toxicity (Kouri et al., 1974; Bradlaw et al., 1975; Bradlaw et al., 1976; Niwa et al., 1975). Knutson and Poland (1980b) examined over 30 HAHs in XB cells, a mouse teratoma cell line which appears to undergo a keratinization in response to TCDD. While the correlation between *Ah* receptor binding and keratinization was very good for these compounds, the effect to produce AHH induction could be dissociated from the "toxic" effect of the TCDD on XB cells.

Three additional lines of evidence weaken the proposal that *Ah* receptor binding is the critical event in the development of HAH toxicity:

1) A number of studies have found a dissociation between inductive effects of HAHs and toxic effects, in addition to the XB cell culture studies mentioned above. Greig et al. (1984) reported that the development of porphyria induced by TCDD in mice does not correlate with the *Ah* phenotype, and Seki et al. (1987) made a similar observation with porphyria induced by PCBs. Rifkind et al. (1984) studied HAH toxicity in a chick embryo model and found that benoxyprofen can prevent the lethality, pericardial edema, and thymic atrophy induced by 3,4,3',4'-tetrachlorobiphenyl without affecting AHH induction. Brunstrom and Andersson (1988) found that while the rank order for HAH toxicity and EROD induction in the chick embryo was the same for three coplanar congeners, the relative potencies among the congeners for toxicity and induction differed.

2) There is a lack of correlation between *Ah* receptor binding and toxicity of HAHs across species. For example, there are enormous differences among species with respect to the sensitivity to TCDD toxicity, covering a 5000-fold range of acute LD50 values. The LD50 values for TCDD in several species are tabulated in Table 3.3.1. Despite this wide range of sensitivities to TCDD toxicity, the binding affinity of TCDD to the *Ah* receptor is remarkably consistent among species. This is illustrated by the binding study of Gasiewicz and Rucci (1984). Data from this study are presented in Table 3.3.2. Thus, this wide range of sensitivities to TCDD toxicity does not correlate to the similar *Ah* receptor binding affinities and *Ah* receptor concentrations found in the tissues of these same species. For example, compare the differences in LD_{50} for various species (Table 3.3.1) to the almost equivalent Ah receptor binding affinities (Table 3.3.2) and tissue *Ah* receptor concentrations (Table 3.3.3) in these same species.

Although there is evidence that the nature of the molecular properties of *Ah* receptors differs among species (Denison et al., 1986; Romkes et al., 1987), this does not account for the lack of correlation between binding affinity, number of receptors, and sensitivity to toxicity.

3) There is a lack of correlation among HAHs between binding to the *Ah* receptor and toxicity within a species, e.g. the guinea pig. The guinea pig is among the most sensitive species to the toxicity of TCDD. As can be seen in Table 3.3.4, there are a number of HAHs which bind to the *Ah* receptor in the guinea pig with affinity similar to TCDD. Yet among these compounds there is a wide range of toxicity, with 30-day LD50 values ranging from approximately 2 to >10,000 μg/kg (Table 3.3.5). For

example, 3-methylcholanthrene binds to the *Ah* receptor in the guinea pig with an affinity similar to TCDD. The lethal dose of 3-methylcholanthrene, however, is approximately 10,000 times that of TCDD. In the mouse, Holsapple et al. (1986) found that 2,7-dichlorodibenzo-p-dioxin, a polychlorinated dibenzodioxin which lacks affinity for the *Ah* receptor, produced immunosuppression similar to that produced by TCDD. Clearly, when all HAHs are considered there is a poor correlation between *Ah* receptor binding and toxic potency.

These problems associated with attempting to correlate *Ah* receptor binding to toxicity have caused McKinney et al. (1985a) to propose that there are at least two receptors for HAHs, and that toxicity is mediated through combination with a receptor other than the *Ah* receptor. Noting that plasma thyroxine levels are diminished in TCDD-treated animals (Bastomsky, 1977), McKinney et al. (1985b) used thyroxine-binding prealbumin as a model for the nuclear thyroid hormone receptor and studied the effects of structure on binding for a limited series of HAHs. The structural requirements for binding the *Ah* receptor, combined with the structural requirements for binding to prealbumin, corresponded to those required to produce toxicity. Based upon these and other observations, the authors hypothesized that the *Ah* receptor functions as a storage and translocating protein which is in equilibrium with nuclear binding events. Within the nucleus, a two-protein activated complex is generated by association of the *Ah* receptor-TCDD complex with a second chromatin-bound protein receptor. Binding to the second protein receptor would be facilitated by lateral halogen interactions similar to those observed for prealbumin binding. Cooperative binding of the two proteins would result in activation of the complex, controlling nuclear events which would lead to the subsequent toxic responses.

McKinney's group extended their observations on binding with thyroxine-binding prealbumin in a study of halogenated biphenyls (Rickenbacher et al., 1986). The PCBs were found to be highly effective competitive binding ligands

Table 3.3.1

Acute Oral LD_{50} Values for TCDD in Various Species

guinea pig	1 μg/kg
rat, male	22
rat, female	45
monkey	<70
mouse	114
rabbit	115
dog	>300
bull frog	>500
hamster	5000

Source: Knutson and Poland, (1982)

Table 3.3.2

Concentration, Apparent Equilibrium Dissociation Constants, and Hill Coefficients of Hepatic Cytosolic Receptors for TCDD in Various Mammalian Species

species	n[a]	K_D	n_H
guinea pig (8)	59 ± 11	0.06 ± 0.02	0.99 ± 0.03
rat (7)	61 ± 23	0.12 ± 0.03	1.01 ± 0.03
monkey (3)	42 ± 8	0.26 ± 0.04	1.02 ± 0.03
mouse			
C57BL/6J (4)	74 ± 10	0.29 ± 0.04	1.02 ± 0.04
B6D2F1 (3)	23 ± 2	0.42 ± 0.03	ND[b]
DBA/2J (3)	---[c]	---[c]	---[c]
hamster (7)	67 ± 22	0.33 ± 0.07	1.03 ± 0.06

n = number of binding sites, K_D = apparent equilibrium dissociation constant, n_H = Hill coefficient.
Values are expressed as the mean ± SD; number of determinations is indicated in parentheses.
[a] femtomoles of [^{3}H] TCDD specifically bound per mg cytosolic protein, assuming one binding site per molecule.
[b] ND, not determined
[c] ---, unable to determine, undetectable.
Adapted from Gasiewicz and Rucci, (1984)

Table 3.3.3
Relative Tissue Concentrations of Receptors for TCDD

tissue	guinea pig	hamster	rat	mouse (C57BL/6J)
	fmoles / mg cytosolic protein			
liver	59 ± 11	67 ± 22	61 ± 23	74 ± 10
lung	86 ± 28	35 ± 20	76	
thymus	47 ± 7	5 ± 6	121 ± 30	24 ± 2
duodenum	18	ND[a]	--	--
kidney	24 ± 19	13	--	--
spleen	17	6 ± 5	--	--
testes	50 ± 7	ND	--	--
heart	16	ND	--	--
pancreas	ND	ND	--	--
muscle	ND	ND	--	--
adrenal	3 ± 3	ND	--	--
brain				
midbrain	ND	ND	--	--
cerebrum	11	14	--	--
medulla	ND	ND	--	--
cerebellum	12	ND	--	--
hypothalamus	ND	ND	--	--

Most values are expressed as the mean ± SD. For those tissues in which N=2, the mean only is reported.
[a] ND, not detectable
Adapted from Gasiewicz and Rucci, (1984)

Table 3.3.4

Approximate Equilibrium Dissociation Constant, K_D, of Halogenated Aromatic and Nonaromatic Compounds that Bind the *Ah* Receptor

compound	K_D, M
Halogenated aromatic compounds	
2,3,7,8-tetrachlorodibenzo-*p*-dioxin	10^{-8}
2,4,6-triiodophenol	10^{-8}
2,3,7,8-tetrachlorodibenzofuran	10^{-7}
3,4,5,3',4',5'-hexachlorobiphenyl	10^{-7}
2,3,4,5,3',4'-hexachlorobiphenyl	10^{-6}
Nonhalogenated aromatic compounds	
3-methylcholanthrene	10^{-8}
benzo[a]pyrene	10^{-8}
isosafrole	10^{-8}
butylated hydroxytoluene	10^{-8}
b-naphthoflavone	10^{-7}

Adapted from McKinney et al. (1985a)

Table 3.3.5

Median Lethal Dosages of Halogenated Aromatic Hydrocarbons in the Guinea Pig

compound	LD_{50-30} (μg/kg)
Compounds of high toxicity	
2,3,7,8-tetrachlorodibenzo-*p*-dioxin	2
2,3,6,7-tetrabromobiphenylene	< 10
2,3,7,8-tetrachlorodibenzofuran	7
2,3,6,7-tetrabromonaphthalene	206
3,4,5,3',4',5'-hexachlorobiphenyl	500
3,4,3',4'-tetrachlorobiphenyl	< 1,000
Compounds of low toxicity	
2,3,7-trichlorodibenzo-p-dioxin	> 29,000
2,3,6,7-tetrachloronaphthalene	>> 3,000
2,2'-difluoro-3,4,5,3',4',5'-hexachlorobiphenyl	>> 3,000
2,3,4,5,3',4',5'-heptachlorobiphenyl	> 3,000
3-methylcholanthrene	> 10,000

LD_{50-30}, Median acute lethal dosage following administration of a single dose and observed for 30 days.
Adapted from McKinney et al. (1985a)

for thyroxine-specific binding sites, and binding activity of the PCB congeners correlated with structure-toxicity relationships. In the discussion, the authors suggest that PCBs may bind to thyroxine-specific binding proteins in blood and alter hormone-protein interactions involved in the maintenance of normal thyroid status. Those PCBs with the appropriate structural requirement, i.e. coplanarity, could produce significant toxicity by concentrating in the nucleus and producing potent and persistent thyroxine-like effects. In another report published in 1986, McKinney and colleagues (Pedersen et al., 1986) described a theoretical binding model for the PCB interaction with human plasma prealbumin.

An important aspect of the thyroxine-binding prealbumin binding studies of McKinney and colleagues was the use of this protein as a model for the thyroxine nuclear receptor. In 1987, McKinney et al. used rat liver nuclear extracts to show that thyroid hormone analogues, PCBs, and related compounds were capable of binding to thyroxine-specific sites. Structure-binding relationships for thyroxine-binding prealbumin were generally similar to those for the nuclear receptor. Binding was not always quantitatively similar, however, and 2,4,6-triiodophenol, 3,4,5,3',4',5'-hexachlorobiphenyl, and 3,5,3',5-tetrachlorobiphenyl had significantly lower affinity for the nuclear receptor than for thyroxine-binding prealbumin. In contrast, 3,3',5,5'-tetrachloro-4,4'-dihydroxybiphenyl and 3,3',5,5'-tetrachlorodiphenoquinone had significantly higher binding in the nuclear receptor. The authors suggest that the low affinities of the former compounds may be due to their relatively high lipophilicity, leading to binding to low-affinity lipophilic sites. The structure-binding relationships for the nuclear receptor supported possible involvement of this receptor in the toxic effects produced by PCBs. The nuclear origin of the receptor suggests that it may function as a gene regulatory protein.

Receptor mechanisms have been used in an attempt to explain interactions between HAHs in producing biological effects. Bannister and Safe (1987) administered 2,4,5,2',4',5'-hexachlorobiphenyl either seven days before or concurrently with TCCD to C57BL/6J and DAB/2J mice. Pretreatment with the hexachlorobiphenyl increased the cytosolic levels of *Ah* receptor in the C57BL/6J

mouse but not the DBA/2J mouse. Receptor affinities were not altered in either strain. Cotreatment of hexachlorobiphenyl and a minimally-inducing dose of TCDD resulted in an apparent synergistic increase in the aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD) in both strains. Hexachlorobiphenyl did not increase AHH or EROD activity produced by a maximally-inducing dose of TCDD in the C57BL/6J strain, but increased these activities in the DBA/2J mouse. The authors postulated that the synergistic effect may be the result of hexachlorobiphenyl-induced increases in the *Ah* receptor levels even though no such increase was noted in the DBA/2J mouse. This study found no evidence of a synergistic effect with respect to the toxic endpoints, thymic atrophy and body weight loss. The only significant effect on toxicity was a protection afforded by hexachlorobiphenyl against body weight loss after TCDD administration in the DBA/2J mouse.

Haake et al. (1987) also found that PCBs are capable of producing some protection against the toxic effects of TCDD. Administration of 20 μg/kg TCDD to C57BL/6J mice caused 61.8% cleft palate per litter without signs of obvious maternal toxicity. Aroclor 1254 (244 mg/kg) produced no cleft palate. Cotreatment of TCDD with Aroclor 1254 (same doses) reduced the cleft palate incidence from TCDD to only 8.2%. These results with Aroclor 1254 are in contrast to those of Birnbaum et al. (1985) who found that although 2,3,4,5,3',4'-hexachlorobiphenyl produced no cleft palate when administered alone (20 mg/kg), co-administration of this dose with TCDD (12 μg/kg on gestation days 10-13) caused up to a 10-fold increase in cleft palate over TCDD administration alone. Haake et al. (1987) propose that these contrasting results may be a consequence of the different relative potencies between the Aroclor 1254 mixture and the relatively toxic specific hexachlorobiphenyl congener. They point out that there are substantial differences between Aroclor 1254 and specific hexachlorobiphenyls in ED50 values for biological effects such as body weight loss, thymic atrophy, and AHH induction. The Aroclor mixture, in competing with TCDD for *Ah* receptor binding sites, may be interfering with the binding and resulting effectiveness (toxicity) of the more potent TCDD. Haake et al. (1987) cite a number of lines of evidence in support of this hypothesis. The antagonism of TCDD teratogenicity by Aroclor 1254 appeared

to be specific. Similar treatment did not antagonize the teratogenicity of dexamethasone.

Another study by Safe and coworkers (Bannister et al., 1987) demonstrated that Aroclor 1254 can also antagonize the enzyme induction and immunotoxic effects of TCDD. The *in vitro* induction of AHH by a dose of TCDD was antagonized by a non-inducing dose of Aroclor 1254 using Aroclor/TCDD concentration ratios of 100:1. Analysis of saturation binding curves indicated that Aroclor 1254 is a competitive antagonist of Ah receptor binding of TCDD. Studies with C57BL/6J mice demonstrated that *in vivo* AHH induction by TCDD was also antagonized (by up to 23%) by co-administered Aroclor 1254, but only at Aroclor 1254/TCDD ratios of 1667:1, 5000:1, and 10,000:1. In addition, it was demonstrated that the T-cell-mediated immunotoxicity of TCDD could be completely or partially blocked by co-administration of nonimmunotoxic doses of Aroclor using Aroclor 1254/TCDD ratios of 1340:1 to 20,160:1. Both *in vivo* induction and immunotoxicity could be reversed by larger doses of TCDD. These results suggest that Aroclor 1254 is a competitive antagonist of TCDD and that biological and toxic effects of TCDD can be completely or partially abolished by Aroclor 1254 within a certain range of doses and dose ratios. It was noted that PCB/PCDD (PCDD = polychlorinated dibenzo-p-dioxins) ratios found in analysis of human tissues and environmental samples are comparable to the Aroclor 1254/TCDD ratios that resulted in antagonism. Thus, it is possible that PCBs may provide some protection from the effects of PCDDs under some environmental conditions.

Another study has suggested that at least one PCB congener (2,2',4,4',5,5'-hexachlorobiphenyl) can increase hepatic *Ah* receptor levels and thereby interact with TCDD by this mechanism (Leece et al., 1987). The interactions between 2,2',4,4',5,5'-hexchlorobiphenyl and three PCB congeners (3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4',5,5'-hexachlorobiphenyl, and 2,3,3',4,4',5-hexachlorobiphenyl) that bind with high affinity with the *Ah* receptor were studied in male Wistar rats. 2,2',4,4',5,5'-Hexachlorobiphenyl, at a dose sufficient to produce an increase in hepatic *Ah* receptor levels, did not significantly alter the thymic atrophy induced by the three congeners; however,

2,2',4,4',5,5'-hexachlorobiphenyl significantly increased hepatic cytochrome P-450 enzyme induction. This observation was considered to be evidence of a synergistic interaction. It was thus concluded that the increased responsiveness of rats was due to elevated *Ah* receptor levels. The unpublished observation that 2,2',4,4',5,5'-hexachlorobiphenyl does not elevate thymic *Ah* receptor levels was offered as an explanation for the lack of a increase in thymic atrophy.

3.3.3 Summary of Mechanisms of Toxicity

Despite considerable study, little has been established regarding the mechanism by which PCBs produce their toxic effects. The metabolism of some PCB congeners has been shown to result in the formation of reactive, covalently-binding metabolites. However, this does not appear to be a likely mechanism of PCB toxicity because structural features of PCB congeners that favor this metabolite formation are those associated with less toxicity. Much attention has been given to a proposed mechanism of toxicity in which the binding of PCBs to a cytosolic receptor initiates the toxicity. Among inbred mouse strains there is generally a good correlation between binding to this receptor, called the *Ah* receptor, and a number of toxic effects of PCBs. However when all species are considered, the correlation between binding and toxicity is poor. Further, within species the correlation between binding and toxicity for a number of halogenated aromatic hydrocarbons is also poor. Thus, while binding to the *Ah* receptor may play a role in some toxic effects of PCBs, it does not appear to be the critical event. It has been proposed that the *Ah* receptor functions as a transport protein, carrying the PCB molecule to the nucleus where it interacts with another receptor to initiate the toxic event. The nuclear thyroxine-binding site has been suggested to be this second receptor, although it is unclear precisely how even prolonged stimulation of this receptor would result in the characteristic PCB effects. It has also been proposed that weakly toxic compounds such as the PCBs antagonize the effects of more toxic halogenated aromatic hydrocarbons such as tetrachlorodibenzo-*p*-dioxin by competing for the *Ah* receptor.

3.4 REPRODUCTIVE TOXICITY

3.4.1 Studies in Mice

In the study of Orberg and Kihlstrom (1973), 23 of 45 female NMRI mice were fed PCBs (Clophen A60, 0.025 mg/day) dissolved in peanut oil. Twenty-five of these animals were used to study the effects of PCBs on the length of the estrous cycle. The authors reported that PCB exposure had significantly lengthened the estrous cycle of mice fed Clophen A60 for 62 days (see Table 3.4.1).

Table 3.4.1

Effects of Clophen A60 on the Estrous Cycle of the Mouse

Group	N	Total number of cycles in 62 Days	Mean length of the cycles (days)
Control	14	120	6.6 ± 2.5
Clophen A60	11	69	8.7 ± 4.3*

* reported as significant, p <0.0005 (Student's t-test)
Adapted from Orberg and Kilhstrom (1973)

At the end of the initial 62-day feeding period designed to measure the effects of PCBs on the estrous cycle, each female was placed with a male. Feeding of PCBs continued until the females were sacrificed on day 8-10 of pregnancy. As can be seen in Table 3.4.2, the authors observed that the percentage of implantations in control mice was about 87% while the implantation percentage in PCB-treated animals was only about 80%, a small but apparently statistically significant difference. In part this difference appears to result from the considerably greater number of corpora lutea observed in the animals fed PCBs and the fact that the number of implantations per mouse in the PCB group was lower, although this latter change was probably not significantly different. As both implantations and the estrous cycle are regulated by sex steroids, the authors felt that the observed effects were most likely the result of a PCB enhancement of steroid

Table 3.4.2

Effects of Clophen A60 on Implantation Frequency in the Mouse

Implantations Group	N	Total number of corpora lutea	Number of implantations (% of ova implanted)	Implantations per mouse
Control	22	288	250 (86.8%)	11.4
Clophen A60	23	313	249 (79.6%)*	10.8

* reported as significant, p <0.025 (Chi Square)
Adapted from Orberg and Kilhstrom (1973)

metabolism. According to the residue analysis, the PCB content of the liver, on a liver fat weight basis, ranged from a low of 44 ppm to a high of 424 ppm in these animals. Control values did not exceed 5 ppm. Unfortunately, the authors failed to provide the mean value for liver tissue and adipose tissue, which would have given a better indication of the final PCB body burdens attained by these animals.

Apparently, an initial study by Torok (1973) found that 2,2'-dichlorobiphe- retarded the development of mice when given during the period of blastogenesis. [Note : This particular article was written in German, and so the introduction of Torok (1976) was used as the synopsis of this study.] In a follow-up study, Torok (1976) gave pregnant NMRI mice either 0 (group I), 375 (group II), or 750 mg/kg/day (group III) 2,2'-dichlorobiphenyl during the first three days following conception. The treatment was associated with an increase in the mean duration of pregnancy (18.2, 19.4, and 21.8 days, respectively) and a decrease in the mean litter size (11.5, 7.9, and 4.8, respectively). No statistics were provided in Table 2 of this paper, but it was indicated in the text that the increase in the duration of pregnancy was in fact significant. As in the previous paper, the authors speculated that the implantation rate was being affected by a PCB-induced increase in sex hormone metabolism.

Orberg and Lundberg (1974) studied the effects of PCBs (and DDT) on seminal vesicle weight in castrated and intact male mice. In intact mice, the seminal

vesicle weights (dry weight) for Clophen A60-treated mice (10 mg/kg/day for 28 days) were the same as controls. Among mice castrated and treated with testosterone, PCB treatment caused a decrease in seminal vesicle weight. The authors proposed that PCB induction of hepatic, steroid-metabolizing enzymes in the castrated, testosterone-treated mice caused them to have lower testosterone levels. Lower testosterone would account for the decrease in seminal vesicle size. In intact mice, compensatory increases in testosterone synthesis could overcome the effects of induction, sustaining normal testosterone levels.

Sanders and coworkers (1977) studied the effects of PCBs in conjunction with nutritional restriction on male reproductive and endocrine function in the mouse (strain unspecified). Sixty male mice were split into two groups of thirty. The first group was fed *ad libitum* for thirty days while the second group was fed one half of the amount consumed by the first group. On day 15 each group was further subdivided; those animals fed *ad libitum* had Aroclor 1254 incorporated into their diets at a level of 0, 50, or 200 ppm, while animals on the restricted-intake diet (fed half the amount of food as the *ad libitum* groups) were fed diets containing twice the concentration of PCBs, i.e. 0, 100, or 400 ppm. The PCB intakes were, therefore, comparable between the *ad libitum* and food-restricted groups. The PCB treatment had no effect on body weight, but did increase liver weight in both groups of animals. The weight of the adrenal glands was significantly increased by the high dose of PCBs and by food restriction. The effects on the liver and adrenals resulted in lower pentobarbital sleep times indicating an increase in hepatic metabolism and an increase in plasma corticosteroid levels. Interestingly, the PCB-induced effects on hepatic metabolism and plasma corticosteroids were greater in the animals on the restricted diet. The authors interpreted this finding to mean that the reduced caloric intake makes the liver more sensitive to enzyme induction. However, as the restricted diet decreased body weight and no doubt fat tissue in these animals, the greater induction might also reflect the higher PCB tissue levels achieved in the leaner animals. The authors further speculated that the increase in plasma corticosteroid levels may be responsible for the reduced resistance to infection reported in some studies. Yet the authors also noted that other studies have reported that PCB exposure reduces plasma corticosteroid levels. PCB treatment had no effect on the tissue weights of the seminal vesicle,

preputial gland, or the testes, while the restricted diet decreased all of these parameters. PCB treatment at the highest dosage did, however, significantly reduce testicular sperm count, an effect observed in an earlier study (Sanders and Kirkpatrick, 1975). As the total reduction in testicular sperm count at the highest dose was only 12-13%, it is not clear whether this change would significantly decrease male reproductive performance.

Orberg (1978) fed pregnant female NMRI mice 0.05 mg/day of either 2,4',5-trichlorobiphenyl or 2,2',4,4',5,5'-hexachlorobiphenyl from day 5 of pregnancy until 22 days post-partum. Less than 18 hours after parturition the number of mice per litter was adjusted to ten. Growth, as measured by increases in body weight, was then measured up to weaning (i.e. day 22), at which time the pups were isolated from the dams. Body weight was then followed an additional 13 days (i.e. until day 35). Both PCB treatments appeared to cause changes in specific growth parameters which were statistically significant (relative growth rate, absolute growth weight, and body weight at the end of the observation period), but the observed changes were inconsistent and small. For example, the largest difference from control in mean body weight for a PCB-treated group was 6%. The largest difference in relative growth rate was 7%. The general trend was for PCB treatment to result in heavier offspring with higher growth rates and thus it does not appear that PCBs had adversely affected these animals.

Mattsson et al. (1981) fed pregnant mice 0.5 mg/day of 2,2',4,4',5,5'-hexachlorobiphenyl (HCB) beginning on day 5 of pregnancy for a period of either seven days (group 1) or 13 days (group 2). Two groups of pregnant CBA mice were used; CBA females mated to CBA males and CBA females mated to NMRI males. PCB treatment had no significant effects on thymus or spleen weights in pregnant mice. In contrast, the liver weights were significantly greater in the HCB-treated mice. The number of implantations per mouse was not affected by the HCB treatment, and the resorption frequency was normal for all groups except in one control group (no PCB treatment) of CBA females mated with NMRI males where the resorption frequency had increased from 6.3% to 17.5%. The mitotic activity of spleen cells, which was used as a measure of immunotoxicity, was slightly greater, but not significantly so, in mice fed HCB.

On the basis of the preceding data, the authors concluded that HCB was not fetotoxic, even at doses capable of inducing metabolism.

In order to examine the tissue distribution of PCBs in pregnant mice and their fetuses, Torok and Weber (1981) administered, by gavage, an unreported amount of radiolabelled PCBs to dams on days 13, 15, or 17 of gestation. The animals were sacrificed on the following day, and tissue distribution was determined by autoradiography and by liquid scintillation counting. Both 2,4',5-trichlorobiphenyl and a PCB mixture containing 30% dichlorobiphenyl and 70% trichlorobiphenyl isomers were tested. The distribution pattern was not greatly affected by the gestational age at which the PCBs were administered, although some temporal changes were noted. The radiolabelled PCBs were found in the organs of a number of the dams. The concentrations followed an approximate rank order of fat > liver = mammary gland > kidney > ovary > muscle = blood > brain. An effect caused by the degree and position of the chlorination on the biphenyl isomers administered was observed in this study, as the PCB mixture produced consistently lower tissue levels than did the 2,4',5-trichlorobiphenyl isomer. Transplacental transfer of PCBs was demonstrated in this study, as radioactivity was detected in the placentas, yolk sac, amniotic fluid, and fetuses. The concentration of PCBs detected in the fetuses increased as the dams neared parturition, a fact that was possibly related to the greater lipid content of the older fetuses.

Vodicnik and coworkers (Vodicnik and Lech, 1980; Vodicnik et al. 1980) have also studied the tissue distribution of PCBs in dams and offspring. The study protocol consisted of injecting female mice with a single radiolabelled dose of 100 mg/kg 2,2',4,4',5,5'-hexachlorobiphenyl (HCB) two weeks prior to mating, and following the tissue levels of HCB through gestation and lactation. No significant differences in HCB concentrations were identified during gestation in the blood, muscle, kidney, adipose, and liver tissues of the pregnant females when compared to the tissue distribution of HCB in virgin female mice. However, at birth there was a transient rise in the HCB levels in liver, adipose, and kidney tissue. The sudden significant increase in HCB tissue levels is difficult to understand, for no significant difference in tissue levels prior to parturition were observed. All

tissues of the lactating females underwent a significant decline in tissue PCB concentrations during lactation, and the rapid post-partum decline in liver and adipose tissue PCB concentrations was reflected in the apparent half-lives of PCB elimination from these tissues. The apparent elimination half-lives from liver and adipose tissue were considerably shorter after birth in pregnant/lactating females but had increased during this same interval in the nonpregnant controls. Thus, lactating females had eliminated virtually their entire body burden some 20 days post-partum while virgin mice had not. It was concluded from this study that PCBs were readily transferred to the neonate via breastmilk. These observations suggest that in mice breastmilk is the major route of PCB exposure and accumulation in offspring born to PCB-containing dams (Vodicnik and Lech, 1980).

Using the same experimental design, these authors also reported on the changes in hepatic metabolism that are associated with the changes in PCB tissue levels caused by pregnancy and lactation (Vodicnik et al., 1980). Pretreatment at the 100 mg/kg dose level did not appear to affect reproductive capacity, litter size, or growth rates of offspring. Among virgin mice, PCB treatment resulted in increased hepatic cytochrome P-450 concentrations and mixed function oxidase (MFO) activities throughout the observation period, while induction of hepatic enzymes did not occur in pregnant mice. In fact, the opposite effect occurred and pregnant mice given PCBs had lower cytochrome P-450 concentrations and MFO activities at the time of birth when compared to corn oil-treated control animals. No differences were detected in cytochrome P-450 or MFO activity at birth between offspring from control and PCB-treated mice. On post-partum days 5-20, however, cytochrome P-450 concentrations and MFO activities were elevated in offspring from lactating, PCB-treated mice. The authors concluded from this study that the offspring were receiving sufficient quantities of PCBs from breastmilk to induce hepatic changes.

The effects on offspring of PCBs received through suckling were also studied by Kihlstrom et al. (1974). The parameter measured in this study was growth rate, as indicated by changes in body weight. Mothers were injected with 50 mg/kg Clophen A60 once a week for three successive weeks. Litter sizes were adjusted to

10, with approximately an equal distribution of males and females in the litters. During the first 16 days of life, offspring from PCB-treated mothers grew more rapidly than offspring from controls. During the next 16 days, the growth rate of PCB-exposed pups was less than controls, and by day 33 no difference in body weight was observed.

Welsch (1985) fed female ICR Swiss mice diets containing 0, 1, 10, and 100 ppm of Aroclor 1254. The batch of Aroclor 1254 used was reportedly free of chlorinated dibenzodioxins. Two different exposure regimens were tested. In one regimen, the mice were fed PCBs beginning on gestational day six. In the second regimen, the mice were fed 90 days prior to mating and until day 18 of gestation, at which time all animals were sacrificed. Based on the measured rate of food intake, the dietary PCB levels corresponded to dosages of 0.125 mg/kg/day, 1.25 mg/kg/day, and 12.5 mg/kg/day. The highest dosage decreased conception rates from 85% to 55%, and the chronically-exposed mice had significantly greater liver concentrations of PCBs. Maternal liver levels on day 18 were 10.33 ppm, 48.13 ppm, and 95.73 ppm respectively, for the three dosages tested. Although PCB treatment increased the liver to body weight ratio in all chronically-fed mice, only those fed the 100 ppm diet had significantly larger livers. Similarly, the only significant elevation in cytochrome P-450 per mg of protein occurred in the high dose groups of both exposure regimens. In contrast, the mice chronically fed either 10 or 100 ppm had elevated aminopyrine demethylase and 7-ethoxyresorufin O-deethylase activities. The ingestion of Aroclor 1254 produced no adverse effects on embryonal or fetal development, and no morphological or skeletal defects were observed. Because cyclophosphamide bioactivation is cytochrome P-450-dependent, it was anticipated that PCBs would increase the teratogenicity of this antineoplastic drug. However, except for a reduction in bone growth, both acute and chronic PCB treatments tended to decrease the teratogenic effects of 10-16 mg/kg doses of cyclophosphamide. Still, it was noted that PCB treatment did not attenuate the cyclophosphamide reduction of bone growth and that chronic PCB treatment appeared to cause further reductions. Thus, this study demonstrated that, except for a reduction in conception at the 100 ppm dose, Aroclor 1254 was without reproductive/teratogenic effects and PCB exposure appeared to increase the detoxification of cyclophosphamide as evidenced by a decrease in the

teratogenicity of this drug.

Darnerud et al. (1986) dosed pregnant C57BL mice with 3,3',4,4'-tetrachlorobiphenyl (TCB) at various stages of gestation and followed the distribution of TCB in fetal tissues using whole-body autoradiography. In the early stages of pregnancy the uptake of radiolabelled TCB by fetal tissues could be visualized, but it was localized to the primitive gut. In dams sacrificed on gestational day nine or later, a strong labeling of the uterine fluid could also be observed. Thus, very little of the radiolabelled compound could be found in fetal tissues during the early stages of gestation. At gestational day 13 the radioactivity was still primarily found in the uterine fluid with some uptake by fetal tissues, although levels were lower than those in maternal tissues. By day 17 of gestation the labeling of fetal tissues was marked in comparison to maternal tissue concentrations. Interestingly, the injection of unlabelled TCB four hours prior to the radiolabelled dose resulted in a decreased uptake of radiolabel by fetal tissues. This suggests that TCB uptake is dependent upon a saturable process. Of additional interest was the finding that no unmetabolized TCB was found in fetal tissues. The major metabolite was 2-hydroxy-TCB, but methylsulphonyl-TCB was also identified.

Linzey (1987) examined the effects of Aroclor 1254 on the reproductive success of wild mice trapped in rural western Pennsylvania. Wild white-footed mice, either those caught or their offspring, were allowed *ad libitum* access to water and commercial food. Aroclor 1254 was added to food at a level of 10 ppm for the PCB-treated groups, and the average food consumption of the PCB-contaminated diet was 0.127 g/g-body weight. In the first experiment, 10 randomly selected male-female pairs were placed on either the control or PCB diet for a period of 18 months. While there were no visible effects on appearance or behavior, autopsies of the PCB-treated animals revealed that gross liver changes such as hepatomegaly and yellowish color were common. Not only was this dietary level toxic to the dams, but as can be seen in Table 3.4.3, the level of PCBs transferred via breastmilk was sufficient to decrease the number of pups surviving for 28 days.

Table 3.4.3

Reproductive Data for Wild, White-Footed Mice Fed 10 ppm Aroclor 1254 for 18 Months

Measurement	Control Mice	PCB-treated Mice
% pairs breeding	60%	70%
# litters/breeding female	8.8 ± 1.20 (6)	9.9 ± 2.27 (7)
birth interval (days)	33.3 ± 1.80 (46)	32.3 ± 1.45 (62)
# young/litter at birth	4.4 ± 0.22 (50)	4.0 ± 0.12 (69)
# young/litter at 28 days	3.6 ± 0.30 (50)	2.6 ± 0.22 (68)*

* $p < 0.01$
Adapted from Linzey (1987)

In a second set of experiments, mice born to the wild animals were paired at 12 weeks of age and then placed on control (15 pairs) or PCB (18 pairs) diets for 15 months. The reproductive success of these mice is provided in Table 3.4.4. As can be seen in this table, the number of breeding pairs dropped considerably in the PCB treatment group, i.e. from 18 to only five. The birth interval, number of young born and number of young surviving were also significantly altered. One possible explanation for the poor reproductive success of these animals was provided by the fact that the incidence of liver toxicity (yellowish liver) was considerably greater in this experiment than in the previous study using parent animals.

In the third set of experiments, offspring to the captured wild white-footed mice were paired for mating and fed the PCB diet at 16 weeks of age. Nineteen male-female pairs were placed in the control group and 20 pairs in the PCB-treated group. The dietary exposure lasted between 7.5-9.0 months. Again there was a larger decrease in the number of pairs breeding in the group receiving

Table 3.4.4

Reproductive Data for Offspring of White-Footed Mice Fed 10 ppm Aroclor 1254 for 15 Months

Measurement	Control Mice	PCB-treated Mice
% pairs breeding	53%	28%
# litters/breeding female	6.5 ± 1.64 (8)	3.8 ± 0.97 (5)
birth interval (days)	33.0 ± 1.06 (44)	44.7 ± 6.20 (14)*
# young/litter at birth	4.8 ± 0.15 (52)	3.6 ± 0.23 (19)*
# young/litter at 28 days	4.7 ± 0.18 (50)	2.0 ± 0.38 (18)*

* $p < 0.01$
Adapted from Linzey (1987)

PCBs. But as in the first experiment, only the survival of the young to 28 days was significantly altered by this treatment regimen (see Table 3.4.5). From these results it appears to be clear that this dietary level does not affect reproduction of adult mice or mice at least 16 weeks old. It does, however, significantly decrease the survival of the pups being nursed by dams fed this dietary level. The data in Table 3.4.4 suggests that reproductive success significantly declines if PCB exposure occurs early, but the lack of confirmatory data in support of this suggestion limits any conclusions reached about reproductive performance other than a decreased survival of the young.

Johansson (1987) studied the effects of PCB administration on the testosterone synthesis in male NMRI mice. Beginning at 5 weeks of age or day 13 of gestation, mice were administered oral doses of either 2,2',4,4',5,5'-hexachlorobiphenyl (4-40 mg/kg) or Clophen A50 (8-160 mg/kg) every 2-3 days for 3-5 weeks. At 10-12 weeks of age, plasma testosterone, testis weight, and *in vitro* testosterone synthesis by isolated Leydig cells were determined.

Table 3.4.5

Reproductive Data for Offspring of White-Footed Mice Fed 10 ppm Aroclor 1254 for 9 Months or Less

Measurement	Control Mice	PCB-treated Mice
% pairs breeding	68%	40%
# litters/breeding female	3.8 ± 0.76 (13)	3.6 ± 1.02 (8)
birth interval (days)	33.6 ± 2.70 (37)	34.1 ± 5.81 (21)
# young/litter at birth	4.6 ± 0.20 (49)	4.6 ± 0.28 (28)
# young/litter at 28 days	2.8 ± 0.30 (45)	0.5 ± 0.21 (24)*

* $p < 0.01$
Adapted from Linzey (1987)

Plasma testosterone levels were found to be highly variable, but PCB-treated mice generally had slightly higher testosterone levels during puberty. Testosterone synthesis was not altered in PCB-treated mice. Hexachlorobiphenyl induced an apparent dose-related increase in testis weight/kg body weight, while Clophen A50 treatment resulted in nonsignificant decreases in relative testis weights. Johansson (1987) concluded that the data suggest neither cell viability nor testosterone biosynthesis of Leydig cells were affected by PCB treatments, and that the increased testis weights in hexachlorobiphenyl treatments may result from increased gonadotropin secretion from defective feedback mechanisms suggested in previous literature.

3.4.2 Studies in Rats

Villenueve et al. (1971a) were among the first to examine the fetotoxicity of PCBs to mammalian species. In these experiments sixty female Wistar rats weighing 175-200 grams were bred and then assigned to one of six groups receiving oral dosages of 0, 6.25, 12.5, 25, 50, or 100 mg/kg/day Aroclor 1254 on days 6-15 of gestation. No treatment-related effects were observed for the number of litters or the average litter size. This treatment neither caused fetotoxicity, increased the incidence of resorptions, nor produced visceral or skeletal anomalies. The only effect observed was a decrease in the mean litter weight at the 100 mg/kg/day dosage. The total litter weight was not significantly differ- from control values. In fact, as can be seen in Table 3.4.6, the average litter s

Table 3.4.6

The Effect of Aroclor 1254 Administered to Wistar Dams on Days 6-15 of Gestation

Dosage (mg/kg/day)	Number females treated	Number of litters	Average litter size	Average litter weight	Resorption sites per litter	Average skeletal anomalies	Average visceral anomalies
0	10	9	10.3	4.80	0.33	0.17	0.46
6.25	10	10	10.7	4.70	1.2	0.10	0.17
12.5	10	10	11.3	4.78	0.40	0.08	0.24
25	10	10	11.4	4.70	0.70	0.09	0.15
50	10	9	11.5	4.56	0.44	0.09	0.28
100	10	7	12.0	4.44*	0.71	0.15	0.00

* $p < 0.05$
Adapted from Villenueve et al. (1971a)

appears to increase with the PCB dosage. While this trend is not believed to be treatment-related, it might provide an explanation for the decrease in mean litter weight observed at the 100 mg/kg/day dosage. As the litter size increases the mean litter weight decreases. The inverse relationship between mean litter size and

mean litter weight probably reflects the fact that as the number of pups increases, the nutrition per pup that the dam is able to provide decreases, resulting in a slightly slower growth rate. For as can be seen in Table 3.4.6, the mean litter size in the 100 mg/kg/day group was almost 20% larger than the control litter size, while the mean litter weight in these larger litters is only 7.5% lower than the control value.

Linder et al. (1974) performed what is still perhaps the most extensive reproductive study in rats to date. All animals used in this study were of the Sherman strain and the PCB mixtures evaluated were Aroclors 1254 and 1260. The single, oral dose LD_{50} (measured by observing survivors for 10-14 days even though the mortality occurred during the first three days) in male weanling rats was 1295 mg/kg with 95% confidence limits of 1136-1473 mg/kg for Aroclor 1254. The lowest lethal oral dose tested was 1200 mg/kg, while a 1,000 mg/kg dosage produced mild diarrhea. The oral LD_{50} for Aroclor 1260 was 1315 mg/kg with 95% confidence limits of 1174-1473 mg/kg. The lowest lethal dosage tested was 1,000 mg/kg, while a dosage of 800 mg/kg produced no observable toxicity. For comparative purposes, earlier work by these investigators (Kimbrough et al., 1972) indicates that adult rats are less sensitive, with oral LD_{50}'s ranging from 4,000 to 10,000 mg/kg. The single dose representing the LD_{50} for intravenously administered Aroclor 1254 was 358 mg/kg in female rats. This route of administration leads to a sudden mortality, i.e. death occurs within 5-110 minutes of PCB administration but animals that survive the first 48 hours appear normal (Linder et al., 1974).

The reproductive studies were performed in the following manner. First, a preliminary one-generation study was performed using dietary levels of PCBs of up to 500 ppm. As the highest dose proved to be too toxic, 100 ppm was selected as the maximal dose tested in the two-generation study. In the two-generation studies one group of rats was fed dietary levels of either 0, 1, 5, 20, or 100 ppm Aroclor 1254, while a second group was fed Aroclor 1260 at dietary levels of 0, 5, 20, or 100 ppm. Because the exposure groups had to be staggered, the investigators used slightly different exposure intervals in some cases. A control group, i.e. a

group fed 0 ppm, was included each time a treatment regimen was initiated, so, for comparative purposes, each generation had several control groups (see Table 3.4.7). The specific control group for each dosage group listed in the table can be determined by finding that control group with the same exposure interval. A similarity in dose interval indicates the animals came from the same treatment group. For example, for all F_{1a} animals there are at least three different control groups, i.e. 0 ppm Aroclor 1254. And yet the first control group listed in Table 3.4.7 actually served as the specific control group for those animals whose parents received either 20 or 100 ppm Aroclor 1254 in their diet for 62 days.

The data has been presented in this manner to illustrate the variability in the results obtained in the non-exposed animals. For instance, while the authors concluded that 100 ppm of Aroclor 1254 for 67 days reduced the percent survival of the pups to weaning age, it can be seen that the 85.9% survival measured in this one-generation study (F_{1a}) does not differ significantly from the 89.2% survival measured in one of the control groups of the F_{1b} generation animals, even though the parent animals of this group were not exposed to any PCBs. The one-generation study with Aroclor 1254 did indicate that dietary levels of 500 ppm (i.e. approximately 37 mg/kg/day) were toxic with 67 days of exposure. Female animals fed this dietary level prior to mating suffered a significant decrease in the number and size of the litters conceived. There was also a substantial increase in the number of stillbirths, and newborns failed to live longer than three days. When the dietary dosage was reduced to 100 ppm, however, there was no significant change in any of these parameters compared to the untreated controls for dietary exposure of 62-67 days (see Table 3.4.7).

If the length of exposure for those animals receiving dietary levels of 100 ppm was increased to 186 days prior to mating, the percentage of pup survival at weaning declined to 68%, a significant decrease. A decrease in body size and in the weight of the pups at weaning was noted in all groups whose parents were in the 100 ppm Aroclor 1254 group. However, this decrease in mean body weight of the pups was not observed in the F_2 generations, and this contradiction raises some concern for the reproducibility of this measured change in the F_1 generation.

Table 3.4.7

Reproductive Effects of Aroclor 1254 in Sherman Rats

Dietary level (ppm)	Parental exposure (days)	Number litters (at weaning)	Litter sizes (at weaning)	Total Number Pups Alive (At birth)	(3 days)	(Weaned)	Pup Survival (% weaned)	Mean body wt (weaned)
				F1a				
0	62	17	11.8	211	205	201	95.3%	38.7
0†	67	7	10.6	89	86	85	95.5%	39.2
0	67	18	11.1	202	200	200	99.0%	38.5
1	67	15	9.1	147	145	145	98.6%	42.3
5	67	17	10.8	184	184	183	99.5%	42.1
20	62	19	11.5	222	220	219	98.6%	38.3
100	62	19	10.3	204	199	196	96.1%	32.9
100†	67	8	8.1	85	78	73	85.9%	31.4
500†	67	0	--	8	0	0	-	-
				F1b				
0†	186	7	11.6	81	81	81	100.0%	37.9
0	188	17	11.1	209	194	189	90.4%	37.1
0	201	18	11.0	222	211	198	89.2%	40.7
1	201	17	9.8	200	194	176	88.0%	38.0
5	201	19	10.2	222	218	203	91.4%	38.9
20	188	18	10.1	188	187	181	96.3%	39.0
100†	186	6	8.0	94	74	64	68.1%**	27.9
				F2a				
0	129	18	12.2	225	221	220	97.8%	35.5
0	125	19	11.5	223	220	218	97.8%	37.4
1	125	15	11.5	175	174	173	98.9%	37.2
5	125	17	11.7	205	199	198	96.6%	35.7
20	129	17	10.1	181	174	171	94.5%	36.8
100	129	4	5.6	36	29	28	77.8%**	35.2
				F2b				
0	274	16	11.3	216	201	191	88.4%	31.9
20	274	12	8.5	115	103	102	88.7%	36.1
100	274	2	3.5	7	7	7	100.0%	38.3

Adapted from Linder et al. (1974)

† Results from the preliminary one-generation study (only 10 females were mated instead of the 20 animals used in the two-generation study)

** $p<0.005$

What does seem clear from this experiment is that while adverse reproductive effects were observed at doses of 100 ppm, 20 ppm represented the no-effect level (NOEL) for the neonatal toxicity observed with higher doses of Aroclor 1254, even though it may have produced a decrease in litter size after 274 days of exposure in the F_{2b} generation.

The studies with Aroclor 1260 indicate that it was less toxic than the lesser chlorinated PCB mixture, Aroclor 1254 (see Table 3.4.8). While at dietary levels of 500 ppm there was considerable fetotoxicity and neonatal death, at the 100 ppm dietary level (i.e. 6.9 mg/kg/day) no effects on reproduction were observed in either the one-generation or the two-generation studies with parental exposure intervals of up to 186 days.

Even though reproduction was not affected at these lower dietary levels, the liver weights of offspring fed 20 ppm of either Aroclor were consistently larger than control values, indicating that the induction of hepatic metabolism had occurred. Although the authors reported that Aroclor 1254 at doses of 1 and 5 ppm produced similar effects in the F_{1a} generation, the fact that no such effect was observed in the F_{2a} generation, i.e. animals exposed to PCBs for a considerably longer period, again raises concern for the reproducibility of those specific observations.

These investigators also examined the effects of PCBs given at high doses during gestation. Their findings, listed in Table 3.4.9, indicate that nine doses 100 mg/kg of Aroclor 1254, when given on days 7-15 of gestation, are not fetotoxic. However, this dosage is toxic to the neonates, as 70% of the pups died prior to weaning. No effects on pup survival were noted when the dose of Aroclor 1254 was decreased to 50 mg/kg, and no effects were observed for Aroclor 1260 at either dosage rate. All litters born to dams exposed to these Aroclors were grossly normal and these doses do not appear to have produced any teratogenicity. Thus, in light of later work by Vodicnik and coworkers in the mouse and by Ringer and others in the mink, it appears that the rat pups in the 100 ppm groups received high doses of Aroclor 1254 via breastmilk, and these high doses resulted in the poor survival rate observed for these pups. Furthermore, while reproductive

Table 3.4.8

Reproductive Effects of Aroclor 1260 in Sherman Rats

Dietary level (ppm)	Parental exposure (days)	Number litters (at weaning)	Litter sizes (at weaning)	Total Number Pups Alive (At birth)	(3 days)	(Weaned)	Pup Survival (% weaned)	Mean body wt (weaned)
				F1a				
0†	67	9	12.2	111	111	110	99.1%	37.0
0	68	19	11.2	223	217	213	95.5%	40.9
0	71	19	11.4	222	218	216	97.3%	33.8
5	71	18	11.8	217	213	212	97.7%	38.5
20	68	17	11.3	201	193	191	95.0%	39.8
100	68	17	11.2	197	192	191	97.0%	38.0
100†	67	8	11.6	93	93	93	100.0%	35.6
500†	67	3	3.3	68	55	26	38.2%*	36.3
				F1b				
0†	186	8	10.3	109	96	93	85.3%	40.9
0	187	17	11.0	189	189	187	98.9%	40.8
0	188	16	9.2	175	160	156	89.1%	36.1
5	188	15	10.6	179	171	170	95.0%	39.3
20	187	16	11.4	191	191	183	95.8%	41.0
100	187	13	10.1	135	131	131	97.0%	39.2
100†	186	5	10.8	55	54	54	98.2%	36.2
500†	186	2	2.3	40	38	14	35.0%	40.7
				F2a				
0	128	18	10.3	153	152	151	98.7%	34.8
0	127	13	11.6	213	212	209	98.1%	38.5
5	127	18	11.6	213	212	209	98.1%	38.5
20	128	19	11.1	215	213	211	98.1%	38.7
100	128	20	9.9	201	200	197	98.0%	38.6

Adapted from Linder et al. (1974)

† Results from the preliminary one-generation study (only 10 females were mated instead of the 20 animals used in the two-generation study)

* $p < 0.05$

Table 3.4.9

Effects of Aroclors 1254 and 1260 Administered to Dams on Days 7-15 of Gestation

Dosage (mg/kg/day	Number females treated	Number litters	Litter size (at weaning)	Total Number Pups Alive (At birth)	(3 days)	(Weaned)	Pup Survival (% weaned)	Mean body wt (weaned)
Aroclor 1254								
0	9	9	12.1	111	110	109	98.2%	37.9
0	10	10	11.8	119	119	118	99.1%	40.6
10	9	9	12.0	109	109	108	99.1%	37.6
50	10	10	12.7	134	131	127	94.8%	30.5
100	9	5	3.6	83	64	25	30.1%*	30.8
Aroclor 1260								
0	12	12	11.5	144	140	138	95.8%	33.9
100	12	11	10.3	140	135	124	88.6%	33.9

Adapted from Linder et al. (1974)

* $p < 0.001$

toxicity was observed at higher doses of Aroclor 1254 with chronic exposure, it should be noted that these same doses were previously reported to be hepatotoxic, with adenofibrotic changes being observed at dietary levels of 100 ppm of Aroclor 1254 (Kimbrough et al., 1972).

Alvares and Kappas (1975) injected pregnant Sprague-Dawley rats intraperitoneally with either 25 mg/kg of 3-methylcholanthrene (3MC), 70 mg/kg of

phenobarbital (PB), or 25 mg/kg of Aroclor 1254 (PCBs) for four to six days and measured the effects of these chemicals on placental and fetal liver drug metabolizing enzymes. Animals were sacrificed on the day after the last injection, the 20th day of pregnancy. Using benzo(a)pyrene hydroxylase as the measurement of liver metabolism, 3MC enhanced activity approximately 20-fold in both placenta and fetal liver; PCBs increased placental activity 10-fold but produced only a 3-fold change in fetal liver; PB pretreatment had no effect on the placenta but increased fetal liver activity 4-fold. The same dosage regimen in lactating dams on days 2-8 postpartum likewise increased the liver microsomal metabolism of the newborn pups. PCBs had the greatest effect, increasing fetal liver benzo(a)pyrene hydroxylase 18-fold, cytochrome P-450 content 3-fold, and N-demethylase activity 2-fold. In contrast to exposure during gestation, 3MC administered post-partum was essentially without effect. PB was as effective in inducing N-demethylase activity as PCB, but was less effective in elevating cytochrome P-450 content and only produced a small but significant effect on benzo(a)pyrene activity. The authors concluded that, even though both PCBs and 3MC are highly lipid-soluble chemicals, 3MC had a greater effect on the fetal-placental unit than PCBs, while PCBs were clearly the most potent inducers of neonatal liver enzymes when exposure occurred through breastmilk. These variations in induction potency suggest that the tissue distribution is considerably different for the two chemicals. The greater induction in neonatal livers is also consistent with the efficient elimination of PCBs known to occur through breastmilk during lactation.

This demonstration that PCBs are transferred via the placenta or in breastmilk was subsequently confirmed by Ando (1978), who measured the chemical transport and kinetics of radiolabelled 2,2',4,4',5,5'- hexachlorobiphenyl (HCB). Pregnant female Wistar rats were injected intraperitoneally with approximately 18 μg/kg of HCB, but the gestational day of the injection is not clear. In this study the HCB levels in the newborn increased with time through day 16 postpartum, when the studies were terminated. The transfer of PCBs via the placenta was negligible and represented only 2.7% of the dose. The movement of PCBs to the newborn via milk was considerably greater, and within 16 days 39% of the initial dose had been transferred from the dam to the pups. The total amount of PCB in the pups increased rapidly and correlated to the rapid changes in body

weight that occur during this stage of life. Similar to those studies in mice already reviewed (Vodicnik and Lech, 1980; Vodicnik et al., 1980), Ando found that the tissue levels of PCBs in the nursing dam dropped dramatically during lactation. This observation is consistent with the large portion of maternal PCB body burden that is transferred to the young during this period.

The ability of PCBs to cross the placenta and their transfer via breastmilk has also been studied by Baker et al. (1977). Wistar rats of both sexes were continuously fed 70 ppm Aroclor 1254 for nine weeks. The PCBs were administered in their drinking water, and the Aroclor 1254 was suspended in an emulsion containing 0.15% Tween 80. This exposure apparently corresponded to an approximate dosage of 6.4 mg/kg/day. All control animals were given tap water containing 0.15% Tween 80. This PCB treatment had no effect on the body weights of the adult animals, the 10-day-old pups, or the 20-day-old pups. This treatment regimen was also without effect on liver weight. PCB treatment did increase cytochrome P-450 content, however, and induction was evident in both sexes of the adult animals as well as in the 10- and 20-day-old pups. The increase in cytochrome P-450 in the newborns reached an approximate ten-fold difference. Induction of fetal livers was not significant, suggesting that PCBs were not crossing the placenta in significant amounts, a finding consistent with the study of Ando (1978). Measurements of tissue PCB levels confirmed this suggestion, and PCBs were detected in newborn pups in significantly greater quantities than were found in fetal tissues. No adverse effects on fertility of the male rats were observed, but the incidence of fetal resorption was reportedly increased by week seven (although no data are provided to substantiate the authors' claim). The authors also proposed that the 2,4,5,2',4',5'-hexachlorobiphenyl and 2,4,5,3',4'-pentachlorobiphenyl isomers were responsible for the induction of MFO activity that was observed ten weeks after exposure had been terminated. They based this conclusion on the fact that these two isomers were the only isomers still found in appreciable quantities at this time.

Gellert (1978) examined the estrogenic activity of Aroclors 1221, 1242, 1254, and 1260 in weanling female rats and found that Aroclors 1242, 1254, and 1260 were without activity. Aroclor 1221, however, when given at a dose of 1000 mg/kg,

induced significant uterine growth within 24 hours of exposure. Although this Aroclor induced an estrogen-like change in the uterus, the estrogenic activity of Aroclor 1221, by comparison, was roughly only one millionth that of the hormone estradiol. Similarly, when the four Aroclors were given to neonates on the second and third days of life, only Aroclor 1221 at a dosage of 2,000 mg/kg (two doses of 1,000 mg/kg on successive days) advanced the age of vaginal opening, induced persistent vaginal estrus, and resulted in anovulation as early as six months of age. Gellert (1978) concluded that there is an association between the estrogenic activity of a PCB and its ability to induce premature reproductive aging. However, the significance of this finding is severely limited by the extremely large dosage required to produce this effect in the neonates (this is about one half the LD_{50} for this species) and the fact that it is specific for Aroclor 1221. It is highly unlikely that human exposure, or animal exposures in the wild, will ever be comparable to the large doses used in these experiments. In a subsequent study (Gellert and Wilson, 1979), it was found that Aroclors 1221, 1242, and 1260 did not induce persistent vaginal estrus or anovulation in the female offspring born to dams fed 30 mg/kg/day of PCBs on days 14-20 of gestation. Thus, it would appear that these two studies (Gellert, 1978; Gellert and Wilson, 1979) do not indicate adverse effects from estrogenic activity at realistic PCB exposure levels.

Spencer (1982) measured a number biochemical effects in pseudopregnant female Holtzman rats fed various dietary levels of Aroclor 1254. The methods section of this article is poor and many important details of the experimental design of these studies were not provided in this paper. It would appear from Table 1 of this paper, however, that the animals were fed PCBs only on days 6-15 of gestation. The reported results are as follows. At dietary levels of 100-900 ppm Aroclor 1254 there was an approximate 30% decrease in uterine and ovarian protein content by day 10 in decidualized pseudopregnant rats. There were also severe declines in uterine glycogen content (day 10) and in the protein content of the placenta (day 16). The reproduction studies demonstrated that these biochemical changes preceded a decline in fetal birth weights, but they did not alter reproductive performance. At dietary levels of 25-900 ppm, Aroclor 1254 did not affect implantation or cause resorptions as measured by the number of fetuses present at day 12 of gestation. However, a decrease in fetal birth weight was

observed at doses of 100 ppm or greater, and the fetal survival rate declined at dietary levels of 300 ppm or greater. These fetotoxic effects were somewhat reflective of the maternal toxicity observed at the higher doses, i.e. maternal body weight gain was decreased with diets of 200 ppm or higher and a reduction in food consumption was noted in rats fed diets containing 300 ppm or more of Aroclor 1254.

Brezner et al. (1984) administered 10 mg/kg/day of Aroclor 1254 orally to female Wistar rats for at least one month. Rats sacrificed at the end of the experimental period had PCB tissue levels of 23.9 ppm in liver, 50.9 ppm in brain, and 240.3 ppm in fat tissue. By comparison, control levels for these tissues were 2.4-4.99 ppm. Four weeks after the exposure had ended, tissue levels had declined to 11.5 ppm in liver, 32.5 ppm in brain, and 150 ppm in fat. As in other studies, the authors demonstrated that PCBs were transferred via maternal breastmilk, and PCB levels of 1163 ppm were measured in the breastmilk received by four-day old pups. PCB treatment was high enough to adversely affect body weight gain. PCB-exposed animals suffered a five gram loss in body weight after 30 days while the control group gained an average of 23 grams during this same period. In animals given this daily dosage of PCBs for six weeks the following adverse effects were observed: 67% of the animals suffered a prolongation of the estrous cycle; there was a decrease in sexual receptivity; a delay in the timing of copulation; vaginal bleeding during gestation; a delay of parturition; and a decrease in litter size. PCB treatment decreased pup survival, and body weight gain in the surviving pups lagged significantly behind that of control animals for at least six months. However, in spite of their poor physical appearance, the PCB-exposed offspring showed no noticeable reproductive irregularities as adults.

A delay in the onset of parturition was also reported to result from 3,4,3',4'-tetrachlorobiphenyl (TCB) treatment (White et al., 1983). In these experiments, Sprague-Dawley rats were given dosages of 3 mg/kg/day of TCB by gavage on days 6-18 of pregnancy. This dose led to a one to two day lengthening of gestation that affected approximately 40% of the litters. TCB exposure also decreased litter size, the mean birth weight of the pups, and subsequent pup survival.

The effects of 3,3',4,4'-tetrachlorobiphenyl in the same treatment regimen (3 mg/kg/day on gestational days 6-18) were also studied by Harris and Bradshaw (1984). When fetal mortality was assessed on day 19, the tetrachlorobiphenyl-treated group had approximately twice the rate of resorptions plus dead fetuses. When mortality was evaluated one day after birth (rate of stillborns plus pups dying within 24 hours) the rate in the PCB-treated group was 2.5-times the control. Average body weight was 3% lower among fetuses in the PCB-treated group on day 19, and approximately 12% lower among PCB-exposed pups when measured one day after birth. Pups from tetrachlorobiphenyl-treated dams examined at one day of age showed histopathological and biochemical evidence of hepatic induction.

Cornwall et al. (1984) examined the effect of fetal position in the uterine horn and 3,4,3',4'-tetrachlorobiphenyl (TCB) exposure on fetal weight in Sprague-Dawley rats. Rats were found to have a convex pattern of weight distribution (heaviest fetuses positioned in the middle with lighter animals found in cervical and ovarian terminal ends of the placenta). The administration of TCB caused a significantly greater frequency of late fetal deaths at the cervical end of the uterine horn, but no treatment-related differences were observed in the relative position patterns for either decreased fetal weight or fetal mortality between horns or between sexes.

The increase in late fetal deaths observed by Cornwall et al. (1984) was also observed by Simmons et al. (1984). Female Sprague-Dawley rats were administered 3 mg/kg/day p.o. of 3,4,3',4'-tetrachlorobiphenyl (TCB) on days 6-18 of gestation. As can be seen in Table 3.4.10, this TCB treatment was associated with a small but significant increase in the average number of implants and in the average number of resorptions at day 19 of gestation (part A of Table 3.4.10). However, there was a considerable increase in fetal death during the next three days as evidenced by the decrease in litter size measured one day postpartum (part B of Table 3.4.10). The late fetal deaths occurred primarily in male fetuses. Thus, the late fetal deaths had reduced the male/female sex ratio from 1.0 to 0.72 by day 21 of gestation and from 0.96 to 0.69 by postpartum day one. Pups born to dams

Table 3.4.10

The Effect of 3,3',4,4'-Tetrachlorobiphenyl Administered to Sprague-Dawley Dams on Days 6-18 of Gestation

A. Prenatal Assessment of Mortality

Dosage (mg/kg/day)	Number of litters	Average number implants	Average number of fetuses (day 19)	Average number of resorptions (day 19)	Percent litters with zero resorptions
0	58	12.3	11.4	0.9	40%
3	29	13.1*	11.3	1.9**	17

B. Postnatal Assessment of Mortality (day 1 postpartum)

Dosage (mg/kg/day)	Number of litters	Total number pups	Average number live pups per litter	Percent litters with zero still births
0	130	1183	9.1 ± 3.7	54%
3	62	250	4.0 ± 3.5**	31%

C. Effect of Treatment on Cumulative Weight in Offspring

Dosage (mg/kg/day)	Day 19 of gestation	Postpartum			
		Day 1	Day 5	Day 10	Day 15
0	2.39	6.78	11.18	18.79	37.50
3	2.29*	5.99**	9.94**	16.38**	34.07**

Adapted from Simmons et al. (1984)

* $p < 0.05$, ** $p < 0.01$

exposed to TCB were also smaller in size and remained smaller for at least the first fifteen days postpartum as compared to the controls.

Takagi et al. (1986) studied the distribution and accumulation of radiolabelled Kanechlor 600 in the JCL-SD strain of rat. The dosage used was 10 mg/kg p.o., administered once a week for five weeks. The animals had excreted 55.8% of the total amount of administered PCBs within the first two weeks after the last dose. The average amount of PCB that had been transferred from the dam to the fetus by gestational day 18 was 0.0031% of PCBs accumulated in the dam. The concentration of PCBs in fetal tissues ranged from 170-350 ppb with the highest concentration located in fetal liver. As in other studies, the largest transfer of PCBs occurred after birth. The percentage of PCBs transferred postpartum was 0.042% on day 1, 0.95% on day 4, 3.21% on day 11, 4.23% on day 18, and 4.9% on day 25. This increased whole-body PCB concentrations in the newborn from 0.6 ppm on day 1 to about 8 ppm by days 11-18. The average concentration of PCBs in rat milk for this period was 1.84 ppm.

Reproductive studies in rats have not been confined to effects on female reproductive function. In the study of Baker et al. (1977), male Wistar rats were fed 70 ppm Aroclor 1254 for nine weeks. This PCB exposure did not alter body weight, growth rate, or fertility and no changes were noted in liver microsomal protein content. Dikshith et al. (1975) examined the testicular effects of PCBs in Sprague-Dawley rats. They found that Aroclor 1254, when administered at a rate of 50 mg/kg/day for seven days, had no effect on chromosomal integrity or the rate of spermatogenesis. Histopathological examination of testes from PCB-treated rats showed the testes to be normal. The only difference observed between control and PCB-treated animals was an increase in acid phosphatase activity of the interstitial tissue.

While the results of the above studies were essentially negative, Sager (1983) found that exposing male rats postnatally to PCBs via breastmilk resulted in a decrease in reproductive performance. Pregnant female rats were administered Aroclor 1254 in doses of 0, 8, 32, and 64 mg/kg on days 1-3, 5, 7, and 9 after birth. Male offspring were mated at 130 days of age and autopsied at 165 days. Adult

reproductive function in males exposed to the higher doses (maternal doses of 32 and 64 mg/kg) was significantly different from control. Specifically, fewer females became pregnant after exposure to these males; the average number of implantations and fetuses were reduced; there was a significantly greater resorption rate; and the proportion of ova implanted or implanted and surviving until autopsy (day 11 or 12 of gestation) was lower. Although their body weights and liver weights were normal, PCB-exposed rats generally had lower ventral prostate weights, lower seminal vesicle weights, higher testes weights, and also appeared to be more reluctant to mate. At autopsy (165 days of age), postnatally PCB-exposed rats still had measurable PCB concentrations in both liver and fat. Fat PCB concentrations were 7.41, 17.58, and 37.29 ppm wet wt for the 8 mg/kg, 32 mg/kg, and 64 mg/kg maternal dosages, respectively. The authors speculated that the effects on male reproductive function were at least in part a consequence of a hypoandrogenic state induced by early postnatal exposure. It was also noted that this postnatal exposure produced a toxic syndrome and fetal deaths at the highest maternal dosages. Sager has recently reported a similar experiment, again using Aroclor 1254 (Sager et al., 1987) in an unspecified strain of rat. The results of the experiment are essentially the same as those of the earlier study and add little new information.

Another recent study of male rat reproductive effects of PCBs in rats was performed by Yeowell et al. (1987). Although these studies did not focus on any particular deficit of reproductive function in PCB-treated rats, it is notable that acute exposure to 50 mg/kg 3,4,5,3',4',5'-hexachlorobiphenyl caused an acute decrease in serum testosterone (>90%) and activity of male-specific cytochrome P-450 2c activity in the liver within seven days after exposure. The serum testosterone decreases seen with hexachlorobiphenyl treatment were thought to be the direct result of decreased hydroxylation activity of this male-specific liver enzyme. The authors observed a decreased mRNA content for this liver enzyme and attributed the decreased P-450 2c activity to a decreased translational efficiency of that mRNA, possibly mediated through the *Ah* receptor or PCB interference with the hormonal regulation of the enzyme. Unfortunately, Yeowell et al. (1987) did not indicate whether the acute decreases in serum testosterone were recoverable, nor whether any actual reproductive deficit was eventually produced.

3.4.3 Studies in Mink

Platonow and Karstad (1973) were the first to report that PCBs might induce reproductive failure in mink. These investigators fed mink a diet consisting of the fat, musculature, liver, and kidneys of two Jersey cows that had previously been fed ten consecutive doses of Aroclor 1254 at levels of 1 or 10 mg/kg/day prior to slaughter. The ground cow parts were then added to a commercial mink food cereal at a ratio of 24% cereal:76% cow to generate two separate diets, one containing 0.64 ppm PCBs and one containing 3.57 ppm PCBs. The 32 mink used in this study were then fed one of the PCB diets *ad libitum* for a total of 160 days beginning two months prior to the breeding season (i.e., from Jan. 7, 1971 until June 17, 1971). Beginning in early March the 12 female animals from each dosage group were caged daily with one of the male animals until evidence of mating had been observed. Semen from each male was then vaginally aspirated for microscopic examination.

The 3.57 ppm dietary level of PCB proved to be quite toxic with five of the 12 female animals dying before they could be bred. In fact, this dietary level was so toxic that 16/16 of the animals exposed to this diet died within the first 105 days. The first animal died after 43 days of the diet, and the mean survival time was only 74 days for female animals and 96 days for male animals. In both dosage groups the male animals tended to survive longer, on the average, than did the female animals. Although the exposed male animals were shown to have motile sperm, only one of the exposed females, a low dose animal, produced a litter. However, all three of the kits born to this litter died the first day. In all other cases, the failure to successfully breed was caused either by the female rejecting the male animal or because the female was too weak to participate.

In comparison to the high dose group, only 2/16 of the animals receiving the 0.64 ppm diet died during the 160-day exposure period. From day 160 until the day they were killed, the low-dose mink were fed the control diet. Of the 14 low-dose animals that survived the 160-day exposure to PCBs, 3 females and one male were killed for examination on days 160, 200, and 242. The remaining mink were finally

sacrificed on day 277. The dying animals had poor appetites and were generally lethargic, emaciated, and very weak. Microscopic examination of the animals revealed that the high-dose animals primarily suffered from weight loss (10/16), intra-abdominal hemorrhage (12/16), yellow liver or liver necrosis (12/16), nephrosis (6/16), brain edema (3/16), and disseminated intravascular coagulation (2/16). In contrast, microscopic examination of the low-dose animals revealed a much lower incidence of these findings: none of the animals suffered from weight loss, brain edema or intra-abdominal hemorrhage; only 1/16 had yellow liver and no liver necrosis was evident; 1/16 suffered nephrosis; and only one animal was found with disseminated intravascular coagulation. No lesions were found in the reproductive organs of either sex.

An analysis of the PCB levels in tissues of the mink fed the 3.57 ppm diet revealed the following average tissue concentrations: 11.99 ppm in liver, 7.12 ppm in kidney, 8.31 ppm in heart, 4.72 ppm in brain, 3.31 ppm in muscle, and 1.8 ppm in blood. The animals fed the lower dietary level of PCBs had considerably more uniform PCB tissue concentrations, and the average concentration of all tissues except blood ranged between 0.97 and 1.74 ppm. This uniformity was also observed in the untreated animals which had inadvertently received low levels of PCBs in their diet (i.e., 0.30 ppm PCBs).

While this study demonstrates that PCBs can cause reproductive problems in mink, several features of this study severely limit the reliability of its results. First, the 101 mink serving as controls (i.e., unexposed animals) had very poor reproductive success themselves, producing only 1.8 kits per female. In contrast, normal reproductive success in mink is four or more kits per female. Second, the commercial control diet was later found to contain PCBs and chlorinated insecticides. While this was proposed as a possible reason for the poor reproductive performance of the control group, the normal histology of these animals, in contrast to the numerous lesions observed in the animals intentionally exposed to PCBs, does not support this contention. Third, analysis of the PCBs in the mink when compared to the gas chromatographic tracing of Aroclor 1254 suggests that the cows had altered the PCB composition considerably via metabolism of the lesser-chlorinated biphenyls. Ringer (1984) has more recently

pointed out that the study of Platonow and Karstad (1973) is not representative of the effects produced by commercial PCB mixtures, citing a similar experience in which prior animal metabolism increased the toxicity of the polybrominated biphenyls fed to mink (Aulerich and Ringer, 1979). Fourth, the lack of gonadal histology and the emaciated condition of the animals suggest that the reproductive failure observed in this study may have been secondary to a severe and lethal systemic toxicity. Therefore, more recently published studies, using diets containing commercial PCB mixtures and control animals which exhibit normal reproductive success, serve as a better basis for determining the actual reproductive toxicity that PCB mixtures might represent to mink.

Aulerich and Ringer (1977) reported the findings of an extensive study that covered six years and examined the effects of PCB-contaminated fish and fish diets on mink reproduction and mortality. In the first phase of this study, 201 mink were fed diets that contained 30% of various species of Great Lakes or marine fish. These studies demonstrated that diets containing Coho salmon from Lake Michigan caused reproductive failure in all mated female mink, while mink fed marine fish diets or diets composed of West Coast salmon suffered no ill effects. Although the text of this paper states that 10 kits were whelped by the 53 female animals fed Lake Michigan salmon, Table 1 of this paper lists only 6 kits as being whelped by the 53 female animals fed Lake Michigan salmon. Whatever the actual number was, apparently all but two of the kits were stillborn, and the two kits that were born alive died within the first 24 hours. No teratogenic effects were observed. In those animals fed similar portions of Coho salmon caught on the West Coast of the United States, no impairment of reproductive performance was noted. Because Coho salmon from the Great Lakes area were known to be contaminated with PCBs and chlorinated insecticides, particularly DDT, these initial studies suggested that the chlorinated aromatic hydrocarbons contaminating these fish might be responsible for the observed toxicities. Subsequent studies adding acetone-hexane extracts from fish taken from various sources demonstrated that the toxicant in Lake Michigan fish was located primarily in the fat and could be extracted by fat solvents. Further analysis revealed Lake Michigan fish contained between 10-15 ppm PCBs, suggesting PCBs might be the actual causative agent.

In the second phase of these experiments, the effects of commercial PCB mixtures added to commercial mink diets were investigated. In an initial experiment, PCB-treated animals were fed either Lake Michigan fish as 30% of their diet or a 30% ocean fish diet to which PCBs were added (the final level of 30 ppm was achieved by adding 10 ppm each of Aroclors 1242, 1248, and 1254). Both diets were too toxic, and 6/15 animals fed the salmon diet and all animals fed the 30 ppm diet died. Thus, it is not surprising that no kits were born to either treatment group. The tissue levels of PCBs in these animals were similar to those reported by Platonow and Karstad (1973). PCB tissue concentrations in liver, kidney, spleen, lung, and muscle ran about 4-6 ppm PCBs in both groups of dying animals while brain tissue contained 11 ppm PCBs. Fat tissue was not collected for analysis because of the emaciated condition of the animals.

Dietary levels of 5-10 ppm of Aroclor 1254 were also found to be too toxic, depressing body weight gain after two months of feeding and producing lethality within the first five months of exposure. None of the PCB-treated females was able to bear young, and the maternal mortality in these two groups ranged from 29-71%. Therefore, in the third phase of these studies additional experiments using lower doses were initiated. In the subsequent and longer feeding study that followed, dietary levels of 2 ppm of Aroclors 1016, 1221, 1242, and 1254 were fed to mink for approximately 10 months from August 8, 1972 through June 1, 1973. The total PCB intake at 2 ppm for this study was estimated to have been approximately 61 milligrams. The results of this experiment are provided in Table 3.4.11. As can be seen in Table 3.4.11, only Aroclor 1254 was sufficiently toxic at this dietary level to cause reproductive problems. The lesser- chlorinated Aroclors were all without effect on reproduction. In fact, the number of kits whelped per female appears actually to have been increased by exposure to the lesser-chlorinated Aroclors even though the maternal mortality in these groups clearly indicates that the dosages being tested were maternally toxic doses. Therefore, this experiment would seem to have identified the NOEL (No Observable Effect Level) for mink reproductive toxicity for these lesser-chlorinated Aroclor mixtures. If the duration of exposure to Aroclor 1254 is reduced to five months (January to May), the NOEL appears to be 1 ppm for this particular Aroclor as this dietary level produced no observable adverse effects on reproductive performance.

Table 3.4.11

Reproductive Performance and Mortality of Female Mink Fed Diets Containing 2 ppm PCBs

PCB	Adult Females	Kits		
	Percent mortality	No. whelped per female mated	Percent kits whelped still alive at 4 weeks	Average birthweight
Control (0 ppm)	0%	4.1	64%	9.9 ± 0.3
Aroclor 1016 (2 ppm)	0%	4.5	57%	9.2 ± 0.3
Aroclor 1221 (2 ppm)	12%	6.3	86%	9.6 ± 0.3
Aroclor 1242 (2 ppm)	12%	5.6	91%	9.3 ± 0.3
Aroclor 1254 (2 ppm)	12%	0.3	0%	5.4

Adapted from Aulerich and Ringer (1977)

Additionally, experiments reported in this paper demonstrate that the reproductive toxicity of PCBs is reversible. Eleven female animals fed the Lake Michigan salmon diet and three females fed diets to which 5 ppm of Aroclor 1254 had been added, all of which had failed to whelp any kits, were returned to a PCB-free diet for a period of one year. After nine months of the control diet the females were again mated to control males. Ten of the 14 females whelped kits, and the average litter size was found to be similar to that of control animals.

Bleavins et al. (1980) studied the effects of PCBs in both mink and a closely related species, the ferret. These animals were fed diets containing either Aroclor 1242 at levels of 5-40 ppm or Aroclor 1016 at 20 ppm for a period of eight months. These investigators observed 100% mortality in both sexes of mink fed diets

containing 20 or 40 ppm Aroclor 1242, and 66.7% mortality in both sexes fed diets containing 10 ppm Aroclor 1242. The mortality in animals fed diets containing 5 ppm Aroclor 1242 (8%) was not any greater than nor significantly different from the mortality observed in control animals (12.5%). Thus, the lethality dose-response curve for chronic Aroclor 1242 ingestion in mink is very steep within the relatively small range of doses tested in this study. Aroclor 1016 was far less toxic to mink. A dietary level of 20 ppm produced only a 25% mortality in female animals and was not lethal to any of the male animals. Ferrets proved to be considerably less sensitive to PCBs. Neither of the diets containing 20 ppm of either Aroclor 1242 or Aroclor 1016 produced any fatalities in the ferret.

There was complete reproductive failure in all mink fed diets containing Aroclor 1242. However, of the doses tested, only the lowest dose, 5 ppm, had not caused significant maternal mortality in earlier experiments. In contrast, 20 ppm Aroclor 1016 did not affect any reproductive parameter in mink (see Table 3.4.12), but kit mortality at four weeks of age was double that of the control group (56%-vs-24%), indicating neonatal toxicity occurs at this dosage. It should be remembered, however, that this dose produced maternal toxicity sufficient to result in 25% mortality in female animals. Similar effects on reproductive

Table 3.4.12

The Effect of Aroclor 1016 on Reproductive Performance in Mink and Ferret

Dose	Number of kits whelped/per female mated	Average gestation (days)	Percentage of kits alive at birth	Average number of live kits whelped
Mink				
0 ppm	16/21	50.2	84.9%	4.9
20 ppm	4/9	51.3	83.3%	6.3
Ferrets				
0 ppm	12/12	41.0	95.9%	9.8
20 ppm	10/12	41.2	95.6%	8.7

Adapted from Bleavins et al. (1980)
* $p < 0.05$

performance were observed in the ferret. Aroclor 1242 caused complete reproductive failure while Aroclor 1016, similar in average chlorine weight to 1242 but containing less of the heavily chlorinated congeners, was without effect on any reproductive parameter (see Table 3.4.12) and produced no increases in early kit mortality. This observation, and the fact that the LD_{50} for Aroclor 1254 is considerably lower than that of Aroclor 1242 (2.627-vs-0.699), suggests that mink are more susceptible to the more extensively chlorinated biphenyl congeners. Based on estimations of food consumption, the total dose lethal to 50% of the mink with chronic Aroclor exposure was 315 mg/kg. It should be noted, however, that as the dosage increased, the estimated dose resulting in mortality also increased. Thus, the calculated LD_{50} for the 10, 20, and 40 ppm Aroclor 1242 diets was 315, 521, and 833 mg/kg, respectively. The authors speculated that this increase in LD_{50} with increasing dose may indicate a dose-related difference in absorption. Using the results of this study and those of Aulerich and Ringer (1977), Bleavins et al. (1980) estimated the dietary LC_{50} for Aroclor 1242 (247 day exposure) to be 8.6 ppm and that of Aroclor 1254 (280 day exposure) to be 6.65 ppm. Thus, the evidence of reproductive failure obtained in this study for Aroclor 1242 is neither a remarkable nor surprising finding.

Differences between mink and ferret in reproductive toxicity can perhaps be explained in part by the observations of Shull et al. (1982). These investigators have reported the effects of PCBs on the hepatic microsomal mixed function oxidase systems of mink and ferrets. Although both Aroclors 1016 and 1242 were examined, only Aroclor 1242 caused marked changes in liver metabolism. Although the cytochrome P-450 content/mg protein was greater in mink and was increased to higher levels by PCB administration than those observed in the ferret, the metabolic activity, as measured by rate of benzo(a)pyrene hydroxylase, ethoxycoumarin O-deethylase, and hexobarbital hydroxylase, was higher in the ferret, particularly the female of the species. Of additional interest was the finding that only cytochrome P-448 was increased (i.e., induction of benzo(a)pyrene hydroxylase/ethoxycoumarin O-deethylase activity) even though Aroclor 1242 induces both major forms of the cytochrome P-450 subpopulations in other species. The authors also reported that because Aroclor 1242 was toxic to mink and not

ferrets, the PCB toxicosis was inversely correlated to AHH induction which is contrary to the *Ah* receptor theory of toxicity that has been postulated by Poland and Glover (1977).

Hornshaw et al. (1983) re-examined the reproductive toxicity produced by feeding PCB-contaminated fish from the Great Lakes to mink and measured the PCB tissue burdens in these mink following chronic exposure. One objective of this investigation was to determine whether or not PCB levels in certain fish species of the Great Lakes had been reduced enough with time to allow mink farmers to use these fish species as feed stock. Of the five species of fish tested, carp were the most toxic and caused complete reproductive failure. As can be seen in Table 3.4.13, the other fish species tested, i.e. sucker, perch, whitefish, and alewife, were essentially without effect on mink reproduction. The number of kits whelped, the average number of kits whelped per female, and percent survival of the kits to four weeks were not significantly different from control values for any of these dietary groups. Further, excluding kits born to females fed perch scraps, the mean birth weights and body weights of the kits born to dams fed fish from the Great Lakes were not significantly different from the control values.

The dietary levels of PCBs in the feed derived from these fish species ranged from 0.21-0.69 ppm when measured as Aroclor 1254 except in the carp meal, which was toxic and contained 1.5 ppm PCBs. A PCB residue analysis of these animals indicated that mink accumulate PCB in adipose tissue to levels that are about 17-38 times the dietary level. Elimination studies demonstrated that mink do metabolize and eliminate PCBs, but at a rate considerably slower than rodent species. The half-life for all PCBs in mink was approximated to be on the order of 98 days or longer and varied considerably among specific congeners. Interestingly, one of the congeners followed in the elimination studies was the heavily chlorinated 2,2',4,4',5,5'-hexachlorobiphenyl, which is fairly resistant to elimination in other species. A total body burden analysis of the mink used in the reproduction studies suggests that mink fed a total of 25 milligrams or less would not have significant reproductive problems, but complete reproductive failure might be expected if mink ingest approximately 56 milligrams of the PCBs contained in Great Lakes fish. This dose is consistent with the toxic dose

Table 3.4.13

A. The Effect of Using Various Great Lakes Fish Species as Feed Stock on the Reproductive Performance of Mink

Dietary group	Number females whelped/per females mated	Number of kits whelped	Average # of kits whelped	Percent kit survival (4 weeks)
Control	9/10	38	4.2	55%
Carp	3/11	0*	0.0*	0%*
Sucker	9/10	25	2.8	40%
Perch scraps	9/11	33	3.7	36%
Whitefish	8/10	32	4.0	28%
Alewife	10/12	53	5.3	51%

B. Mean Body and Litter Weights of Kits Whelped and Nursed by Female Mink Fed Fish Products

Dietary group	At Birth		At 4 Weeks	
	Mean body weight (g)	Mean litter weight (g)	Mean body weight (g)	Mean litter weight (g)
Control	8.3	29.7	122	369
Sucker	8.7	24.0	111	185
Perch scraps	8.1	29.7	98*	295
Whitefish	8.5	34.1	107	321
Alewife	8.4	44.5	124	420

Adapted from Hornshaw et al. (1983)

* $p < 0.01$

determined in the study of Aulerich and Ringer (1977), where Aroclor 1254 or the PCBs contained in Lake Michigan fish caused complete reproductive failure when the total amount ingested reached 61-90 mg of PCBs. These PCB dose estimates may be limited by the additive effects of other contaminants found in fish from the

Great Lakes system. Fish from the Great Lakes contain a number of organochlorine compounds (e.g., DDT) whose toxic contribution to the reproductive problems experienced in these mink studies would act to lower the estimated safe and toxic doses of PCBs.

Ringer (1984) recently reviewed the toxicology of PCBs in mink and provides several important additional facts that were not available in the preceding reports. First, as has been previously demonstrated in mice and rats, even though there is some placental transfer of PCBs to the fetus, kits receive far more PCBs during lactation via their milk than they do during gestation (Table 3.4.14). This helps explain why PCB doses that do not alter reproductive success and are not fetotoxic still produce considerable kit mortality during nursing. Thus, early neonatal mortality is related to neonatal PCB exposure rather than prenatal PCB exposure. Second, measurements of placental PCB transport in mink and ferret demonstrate that significantly lower PCB concentrations are achieved in fetuses of the ferret. The author speculated that perhaps this occurs as a result of the greater placental surface area between mother and fetus in mink, allowing for greater transplacental exchange to occur. However, since most of the PCBs transferred to the fetus/neonate occurs postnatally rather than prenatally, the role played by transplacental exchange of PCBs in the considerable difference noted in PCB toxicosis between these two species remains unclear. Third, Ringer points out that the study of Platonow and Karstad (1973) is not representative of the effects produced by commercial PCB mixtures, and cites a similar experience in which prior animal metabolism increased the toxicity of the polybrominated biphenyls fed to mink (Aulerich and Ringer, 1979).

Bleavins et al. (1984) administered radiolabelled Aroclor 1254 to three groups of ferrets. One group consisted of six unbred females; the second group consisted of four pregnant females dosed on day 14 of gestation (first trimester); and the third group consisted of four pregnant females dosed on day 35 of gestation (third trimester). Because the apparent elimination of PCBs was the same in all groups, the values for all animals were combined to yield the elimination curves derived in this study. The total percent of dose absorbed was estimated to be 85.4% of the administered dose. The authors suggest that the measurements of PCBs in the

Table 3.4.14

PCB Concentrations at Birth and 2 Weeks of Age in Kits Born to Female Mink Intraperitoneally Dosed With ^{14}C Aroclor 1242

Animal Number	Biphenyl Concentration[a,b]				
	Newborn Kits		2 Week Old Kits		
	Per Gram	Per Kit	Per Gram	Per Kit	Milk
E-2030	0.012	0.095	0.026	1.463	0.029
F-262[c]	0.009	0.079	–	–	--
F-754	0.008	0.083	0.014	0.745	0.023
F-1312[c]	0.015	0.125	–	–	--
F-1650	0.007	0.064	0.018	1.485	0.023
Mean ± S.E.	0.010 ± 0.0015	0.089 ± 0.010	0.019 ± 0.0035	1.231 ± 0.243	0.025 ± 0.002

Adapted from Ringer (1984)

[a] Concentration is expressed as a percentage of the initial maternal dose per gram of tissue
[b] Value is the average PCB concentration based on two kits per female
[c] No kits surviving to 2 weeks of age

kits born to the pregnant animals indicates that more PCBs cross the placenta if the exposure occurs in the third trimester (0.04% of the dose) than if it occurs in the first trimester (0.01% of the dose). However, the longer elimination period in the latter group may explain these small differences, for Table 1 of Bleavin's paper lists a smaller percentage of the dose in neonates from this group exposed to PCBs via milk for one week. This study further emphasized the observation that most of the neonatal accumulation of PCBs comes from milk rather than transplacentally, and the authors calculated a 1:15 ratio for the placental: mammary transfer of PCBs.

One of the more recent studies of the effects of PCBs on mink reproductive performance has been performed by Aulerich et al. (1985). In this study 90 dark

female mink were administered either 2.5 ppm of Aroclor 1254, 0.1 or 0.5 ppm of 3,4,5,3',4',5'- hexachlorobiphenyl (345-HCB), 2.5 or 5.0 ppm of 2,4,5,2',4',5'-hexachlorobiphenyl (245-HCB), or 2.5 or 5.0 ppm of 2,3,6,2',3',6'-hexachlorobiphenyl (236-HCB). The PCBs were administered as contaminants of the diet, and female mink were fed these diets for one month prior to breeding and on through parturition. The 345-HCB exposure proved to be particularly toxic to the mink. All of the animals fed diets containing 0.5 ppm of 345-HCB died within the first 60 days and 50% of those exposed to 0.1 ppm died within the first 3 months of exposure. The symptoms of 345-HCB treated animals that died included hair loss, anorexia, gastric ulcers, bloody stools, degenerative changes of the kidneys, pancreatomegaly, and ascites. There was a general trend of lower body weights for all PCB-treated groups, but body weights differed significantly from control values only in the high-dose 236-HCB (5.0 ppm) group and in both 345-HCB-treated groups. Dietary exposure to either 245-HCB or 236-HCB had no effect on reproductive performance as measured by pregnancy rate, the period of gestation, the number or mortality of kits whelped, and litter size (see Table 3.4.15). Dietary exposure to Aroclor 1254 (2.5 ppm), however, had marked reproductive effects--only one of ten females mated and whelped, giving birth to one stillborn kit. None of the high dose (0.5 ppm) 345-HCB survived to mate, and none of the low dose (0.1 ppm) 345-HCB treated females still alive during the mating period whelped.

Measurements of cytochrome P-450 and related enzymatic activity were performed on these animals. Because benzo(a)pyrene hydroxylase was significantly elevated in both dosage groups fed 245-HCB, the correlation between AHH activity and toxicity was once again absent in mink. These investigators also measured serum 17-ß-estradiol and progesterone levels in these animals. The only change worth noting was the significant decrease in progesterone levels measured in females fed 2.5 ppm Aroclor 1254. The lower progesterone levels in these animals may have been insufficient to maintain pregnancy, and therefore may provide an explanation for the poor reproductive performance and number of resorptions observed during necropsy of these animals. Lastly, the results of this study also reinforce previous observations that both the general toxicity to mink and the effects on reproductive performance are greatly influenced by the positions of chlorination. The estimated total intake of 345-HCB producing lethality in these

Table 3.4.15

The Reproductive Performance of Mink Fed Various Dietary Levels of Different PCB Congeners

Dietary group	Number females whelped/per females mated	Average gestation (days)	Litter size per female at birth	Average kit body weight at birth
Control				
0 ppm	19/20	48.6	5.5	10.1
Aroclor 1254				
2.5 ppm	1/10	50.0	--	--
245-HCB				
2.5 ppm	8/10	48.5	3.9	9.3
5.0 ppm	10/10	48.4	5.2	10.3
236-HCB				
2.5 ppm	9/10	49.1	5.4	10.0
5 ppm	8/10	51.6	5.4	9.6
345-HCB				
0.1 ppm	0/8	--	--	--
0.5 ppm	--	--	--	--

Adapted from Aulerich et al. (1985)

animals was 3.1 mg for the 0.5 ppm 345-HCB group and 0.9 mg for the 0.1 ppm 345-HCB animals.

Aulerich et al. (1986) examined the effect of PCB metabolism on the toxicity of PCBs to mink. Comparisons of body weight changes and mortality indicated that comparable dietary levels of PCBs derived from rabbit carcasses are more toxic to mink than commercial PCB mixtures. These results confirm earlier observations that metabolism of PCBs by other animals renders the PCB mixture contained in the animal more toxic to mink. Thus, the bioaccumulation of PCBs in prey animals can not be strictly compared to studies in mink using commercial forms of PCBs.

Based on earlier studies involving PCB exposures of a more chronic nature, Hornshaw et al. (1986) measured the LC_{50} concentration for Aroclor 1254 in mink for 28- and 35-day exposures. After three tests the 28-day LC_{50} had been measured

to be 83, 84, and 79 ppm. In tests in which a 28-day dietary exposure interval was followed by a 7-day withdrawal period, considerably lower LC_{50} estimates of 58, 47, and 49 were obtained. Based on the specific criteria used in these tests, these investigators found that the age of the animal, composition of the diet, season, and year had little effect of the outcome of these tests. As has been shown in previous studies with mink, the amount of PCBs necessary to produce toxicity is inversely related to the duration of exposure.

Wren and coworkers (Wren et al., 1987a&b) have examined interactions between PCBs and methylmercury (MeHg) on mink. In the first study, mink were fed diets containing either 1.0 ppm of Aroclor 1254, 1.0 ppm of mercury, or 0.5-1.0 ppm each of both compounds from 12/4/84 to 6/6/85. Due to an unexpected mortality of the mink fed 1 ppm methylmercury, attributed to a synergistic reaction between MeHg and cold stress, this group and the PCB+MeHg group were fed the experimental diets every other day from 2/23/85 to 6/6/85 (Wren et al., 1987a). PCB treatment produced no adverse effects on thyroid function (serum T_3/T_4 levels) or on the morphology of the adrenal and pituitary glands. PCB treatment did, however, decrease the methylmercury-induced lethality observed in female mink exposed to these dietary levels of methylmercury. PCB treatment was also without effect on adult fertility, percentage of females whelped, or the number of kits born per female. These investigators reported that PCB treatment decreased the growth rate of the kits, and that there was an apparent synergistic decrease in kit survival observed in kits from the 1.0 ppm PCB+MeHG group but not in the group receiving lower combination doses of these two chemicals (Wren et al., 1987b). Thus, this later paper confirms earlier work that indicates 1 ppm is the NOEL for reproductive toxicity in mink if the exposure period to Aroclor 1254 is approximately six months or less in duration.

In a follow-up study of the toxicities produced by various PCB congeners, Aulerich et al. (1987) fed mink diets containing either 0.01 or 0.05 ppm of 3,4,5,3',4',5'-hexachlorobiphenyl (HCB) for 135 days. The investigators found that mink fed 0.05 ppm HCB for 135 days suffered a 50% mortality while no animals in the 0.01 ppm group died. However, there was a 20% mortality in the control animals during these experiments which raises some question concerning the

magnitude of the toxicity produced by HCB. That is, was the mortality observed in the high-dose group the result of HCB itself, or did it merely augment an existing problem? Animals fed 0.05 ppm HCB did undergo a significant weight loss during this period, and even the 0.01 ppm group suffered some weight loss, although this change was not significant. The signs of toxicity common to the mink, i.e. anorexia, bloody stools, ascites, and ulcers, were noted in the affected high-dose animals upon necropsy. The high dose of HCB significantly increased organ weights in the brain, liver, kidney, and adrenals of the exposed animals while producing significant decreases in thyroid function, as measured by serum T_3 and T_4 levels. By comparison, the 0.01 ppm dose of HCB increased liver weight and decreased serum T_4 levels, but did not affect serum T_3 levels of the exposed animals. Using food consumption figures, the authors calculated that 0.74 mg of HCB represented the LD_{50} dose, which is consistent with the 0.94 mg calculated from an earlier study (Aulerich et al., 1985). Of additional interest was the fact that the toxic dose did not elevate benzo(a)pyrene hydroxylase activity, which again contradicts the *Ah* receptor theory proposed as the mechanism of toxicity for PCBs and related compounds.

3.4.4 Studies in Monkeys

Allen et al. (1974a) fed six female rhesus monkeys, ranging in age from 7-10 years, Aroclor 1248 at a dietary level of 25 ppm for two months. During this two month period five of the animals (average weight of 5.6 kg) ingested approximately 260 mg of PCBs. For reasons that were unexplained, the sixth animal ingested an estimated 450 mg of PCBs. Within one month the monkey with the highest consumption developed facial edema and hair loss. Similar but less severe symptoms were noted in the other PCB-exposed monkeys after six weeks. The monkey ingesting the greatest amount died two months after the dietary exposure period ended. The cause of death was linked to gastric ulcerations leading to limited food consumption and a 35% reduction in body weight (i.e., decline from 5.9 to 3.9 kg). Complete blood counts and serum chemistry were taken for each animal monthly after the exposure ended. Major hematological changes occurred only in the animal that died. These consisted of significant decreases in total serum protein, albumin, total lipid, and cholesterol with an increase in SGPT

activity. With the exception of the last test, all of the decreased blood parameters may have been related to the anorexia and substantial weight loss suffered by the dying animal. At autopsy the major gross findings in this animal were alopecia, subcutaneous edema, acne, and acute gastritis of the entire stomach lining. Ulcers and hemorrhage were also prominent features in the stomach, and these changes undoubtedly contributed to the anorexia and ultimate weight loss. All remaining tissues were normal with the exception of the bone marrow which was pale and semi-fluid in appearance. Microscopic examinations of the liver revealed necrosis with acute inflammatory response and enlarged hepatocytes with lipid accumulation and eosinophilic cytoplasm. Microscopic examination of the bone marrow showed a small number of red cell precursors. Histology of all other tissues examined appeared normal.

Even though the PCB diet was removed after two months, the condition of the surviving animals deteriorated during the following months. By month four, two of the remaining animals had eyelids so swollen that only a small slit-like opening existed between the eyelids. The PCB adipose levels in these animals averaged 127 ppm after the two-month feeding interval. Eight months later these levels were still 34 ppm, with the animals continuing to show signs of chemical intoxication. A somewhat contradictory finding in the PCB tissue studies was the fact that the animal that died had considerably lower tissue levels than the survivors. For this reason no conclusions can be drawn from this experiment concerning the tissue concentrations that result in lethality in this species.

All surviving animals maintained a regular menstrual cycle, and three months after they were removed from the diet attempts were made to mate these animals. Three of the five animals were thought to have conceived, but only one delivered an infant. During the next five months the nonpregnant animals were bred again without success. The single infant was well developed but its birth weight was lower than normal (i.e. 375 g versus 544 g). This infant was sacrificed for PCB tissue analyses, and these revealed significant PCB concentrations in fat (27.7 ppm) and adrenals (24.4 ppm) only. By comparison the fat and liver concentrations in the infant's mother were 56.3 and 50.0 ppm, respectively.

In a second set of experiments which were similar to those reported in preceding paragraphs, Allen and coworkers (Barsotti et al.,1976) decreased the dietary level but extended the duration the animals were fed PCBs. In this study, 18 female and 4 male rhesus monkeys were fed one of two PCB-contaminated diets, one containing 2.5 ppm Aroclor 1248 or one containing 5.0 ppm of this Aroclor. All of the animals were fed these dietary levels of PCBs for an undisclosed period of time, but it appears from the results section of this paper that they continued on the diet well past the seven-month exposure interval used to test the effects of Aroclor 1248 on their reproductive performance. During the first six months the daily PCB intake by female animals remained relatively constant and corresponded to a total ingestion of 90 and 180 mg of PCBs. Even though food consumption remained within normal limits, both levels of exposure resulted in an average weight loss of 15%. Further, after only two months of the diet some animals began to develop signs of chemical intoxication including acne, hair loss, erythema, and swelling of the eyelids. After an eight-month exposure period, these animals had also developed a hypolipidemia and an elevation of SGPT levels suggestive of some liver involvement. PCB tissue analyses indicated that body burdens reached a plateau after six months in the 5.0 ppm group and after one year of exposure in the 2.5 ppm group. At the six-month exposure interval, PCB levels in fat tissues averaged 32.69 ppm (2.5 ppm diet) and 71.3 ppm (5.0 ppm diet).

After four months of PCB exposure and a consumption of 60-120 mg, menstrual cycles were clearly affected, particularly in those on the 5 ppm diet. These investigators reported observing menostaxis, menorrhagia and amenorrhea in the female animals fed 5 ppm. In addition, one female animal in the 2.5 ppm group died after 173 days and one female animal from the 5.0 ppm diet died on day 310. PCB tissue levels in the latter animal were generally 10-20 times those of the first animal to die, so once again there is no indication of those tissue concentrations which result in lethality. Despite the obvious maternal toxicity of the dosages used, attempts were made to breed these animals after seven months of exposure. Using chorionic gonadotropin levels as an indication of conception and pregnancy, it was believed that all eight animals fed the 2.5 ppm diet had been able to conceive. However, three animals resorbed their fetuses shortly after conception, and normal implantation bleeding was replaced by profuse uterine

hemorrhage. The remaining five animals had normal births. The conception rate of the group fed 5.0 ppm was reduced to six out of eight animals (75%), and the remaining two animals were bred five times without success. Three of the six conceptions in this group ended in abortion after 46-107 days of gestation. One resorption and one stillbirth also occurred, leaving only one live infant born to this group. Residue analysis of the stillborn infant demonstrated that considerable concentrations of PCBs had been accumulated transplacentally (e.g. 63.85 ppm pancreas, 99.36 ppm lung, and 24.85 ppm adrenals), and the levels found in this infant were similar to those of the female adult animal from the 5.0 ppm diet group that died after 310 days of exposure.

All living infants were small but otherwise normal. As in other species, the levels of PCB accumulation in the infants were increased considerably by breast feeding. In the low-dose group adipose PCB levels at birth were only 2.8 ppm, but consumption of breastmilk containing 275 ppb PCBs resulted in adipose levels of 111.6 ppm after three months of breast-feeding.

Adult male rhesus monkeys were also fed the 5.0 ppm PCB diet in this study. Toxicity was considerably lower in these males. While one monkey had signs of periorbital edema and erythema of the eyelids after six months (a 300 mg exposure), the remaining males had developed only minor symptoms after 14 months (670 mg) of exposure. Even after the longer exposure period (14 months), no abnormalities in sperm count or breeding success were observed in the male animals.

Allen and Barsotti (1976) published another report in the same year. Much of the data are identical to those in their previous report (Barsotti et al., 1976), and, while it is not made clear, this report of an 18-month dietary PCB exposure appears to be a follow-up of the same animals used in the previous report. One discrepancy between this and the previous report occurs in the exposure interval prior to breeding, which is listed in this article as having been only six months. This discrepancy appears, however, to have been a typographical error for the seven-month interval prior to breeding that was actually used (Allen et al., 1979). Thus, the female animals were reportedly kept on their respective 2.5 or 5.0 ppm

PCB diets for six months prior to breeding, all through gestation, and for three months after parturition, at which time they were placed on control diets. As in the previous study, 5/8 animals in the 2.5 ppm group gave birth to live infants while 4/6 conceptions in the 5.0 ppm group ended in abortion. The stillbirth occurring in the high dose group is now listed as dying from hypoxia resulting from a difficult delivery, and all tissues were reported to be free of lesions. That this report is a continuation of the previous study is supported by the fact that the tissue PCB levels in the dead offspring are identical to those listed in the previous article (Barsotti et al., 1976).

All of the offspring but one were weaned at the age of four months, and this latter animal remained with its dam for a total of nine months. Milk samples taken from three of the dams on the 2.5 ppm diet contained PCBs ranging in concentration from 0.154-0.397 ppm. A milk sample from the fourth dam contained 16.44 ppm PCBs in milk fat. Skin biopsies revealed PCB levels of 1.0-4.8 ppm at birth, and these levels had risen to 86.4-136.8 ppm only three months after birth. At birth and for the following 12 weeks the body weight of the offspring exposed to PCBs remained considerably lower than their unexposed controls, and the PCB offspring were smaller in stature. Their appearance at birth was normal except for focal areas of hyperpigmented skin. Within two months they began to develop acne, facial edema, loss of eye lashes, and increased pigmentation of the skin. Three of the six offspring exposed to PCBs died within the first eight months. Pathological findings in the dying offspring included thymic atrophy, small spleens, thickening of the gastric mucosa in some animals, and a hypocellularity of the bone marrow. In addition, the livers showed moderate fatty infiltration and one of the animals had developed areas of necrosis and inflammatory cell infiltration. Once the three surviving offspring were placed on a synthetic replacement diet, there was a rapid improvement in their appearance, indicating that the PCB-induced effects were reversible. Skin and subcutaneous fat samples taken at eight months of age, i.e. four months of replacement diet, still contained 19.7 and 4.2 ppm of PCBs, respectively.

One year after their removal from the PCB diets, the female monkeys used in the study reported in the two previously-cited Allen papers were again bred to

control males (Allen et al., 1979). All animals conceived, and 7/8 animals from the 2.5 ppm group and 5/7 of the 5.0 ppm group gave birth to live infants. The infants born to mothers previously fed PCBs were smaller, did not grow as rapidly, and suffered a greater frequency of illness than those born to control animals. Two of the infants born to mothers in the 5.0 ppm dietary group died. In these two animals the thymus was reported as small, while the lymph nodes and spleens were reported as rudimentary. Thus, it appears that monkeys are not only quite sensitive to PCBs, but that some of the adverse reproductive effects disappear slowly. In this article Allen also mentions briefly a study in which monkeys were fed Aroclor 1248 at levels of 0.5 and 1.0 ppm three times a week for seven months prior to breeding. The total PCB dosage at this time was 8-16 mg and it is not clear in this citation (Allen et al., 1979) if exposure was continued throughout gesta- and to weaning as in previous studies. All animals conceived, and 13/16 female animals gave birth to live infants. These infants were somewhat smaller at birth, gained weight less rapidly, and developed areas of focal hyperpigmentation.

Apparently, the effects of the one-year withdrawal period on female animals fed Aroclor 1248 for 18 months was also reported in another article by Allen (Allen et al., 1980). The total PCB intake for animals fed the 2.5 ppm diet was 270 ± 25 mg of Aroclor 1248 and 498 ± 50 mg for animals fed the 5.0 ppm diet. The PCB levels in the subcutaneous fat of these animals are provided in Table 3.4.16. The variability in the last two samples, taken 6 and 12 months after exposure to PCBs ceased, is

Table 3.4.16

Subcutaneous Fat PCB Levels in Monkeys Fed Aroclor 1248 for 18 Months

PCBs in Diet	PCB in Fat (ppm) by Exposure Interval (months)					
	1	6	12	18	24	30
2.5 ppm	5.1	32.7	49.5	117.6	0.7 ± 1.1	0.8 ± 0.9
5.0 ppm	14.0	7.1	109.4	81.1	12.1 ± 14.4	1.9 ± 1.4

Adapted from Allen et al. (1980)

noted by the large standard deviations (or S.E.M., as the text makes no distinction) following the mean values. The recovery period was marked by an improvement in the appearance of the female animals. Normal menstrual cycles and progesterone levels were re-established, and the animals no longer experienced a high incidence of early abortions. After 18 months of PCB exposure, the adipose PCB levels for the groups fed 2.5 and 5.0 ppm PCB averaged approximately 81 and 118 ppm, respectively; at 24 months (six months into the recovery period) these levels had declined to 0.7 and 12 ppm; and at 30 months the levels were only 0.8 and 1.9 ppm (see Table 3.4.16). However, the considerable variability evident in the last two measurements of Table 3.4.16 suggest that some animals had almost no PCBs while others had approximately two or more times the mean values listed.

Despite the considerable improvement in the PCB tissue levels, reproduction was still affected. In the 2.5 ppm group 8/8 conceived and 7/8 carried to term, but 2/7 of the infants later died. In the 5.0 ppm group, 7/7 conceived, only 4/7 gave live births and 2/4 of these infants later died. Histological examinations of the neonates that died revealed a hypocellularity of the thymus and small spleens with a paucity of lymph nodes. The birth weights of offspring born to the 5.0 ppm PCB-treated females were significantly less than those of the control animals and of the 2.5 ppm PCB-treated animals. Analyses of breastmilk and skin biopsies of the infants demonstrated the infants were still being exposed to PCBs. At weaning, the breastmilk contained 50 ppb (50 ng/g) of PCBs on a whole milk basis while infant skin biopsies at birth and three months of age revealed PCB levels increased from non-detectable levels to an average of 3.31 ppm.

The authors noted that the improved reproductive performance of these animals corresponded to a return to normal menstruation and a normal pattern of serum progesterone. However, these investigators concluded that healthy appearing nonhuman primates are capable of carrying sufficient PCBs in their tissues even after the exposure has been discontinued to produce severe health effects in the offspring (Allen et al., 1980). It might also be suggested from these data that after high PCB exposures for a considerable period of time, the females had accumulated sufficient quantities of the more toxic and slower metabolized isomers such that the body burdens were no longer an accurate indication of the

hazard present, or that some permanent, adverse health effects, as noted by the still lower birth rate and early neonatal deaths, had been created by the subchronic exposures these female animals had experienced.

In addition to the work by Allen and coworkers already described, research by Bailey et al. (1980) has stressed the importance of PCB transport via the milk to the infant. Administration of 16 mg/kg/day of Clophen A30 for 30 days to lactating mothers resulted in PCB concentrations in the milk that were approximately 20 times the maternal serum levels. After two weeks of exposure to this milk, infant serum levels were two to five times those of the mother. Tissue analysis of a mother-infant pair, sacrificed when severe symptoms of poisoning became manifest (after 23 days of PCBs), showed that PCB tissue levels were indeed higher in the infant than the mother. This infant's adrenals and liver contained 973.6 and 115 ppm, respectively. The mean tissue PCB concentrations in all sacrificed infants were 333 ppm in the adrenals, 192 ppm in body fat, and 41 ppm in the bone marrow, thyroid, and liver. These findings are consistent with those of Allen, and indicate that the mother's exposure results in a considerably greater PCB exposure and risk to the infant.

A preliminary study examining the reproductive toxicity of Aroclor 1254 in pregnant cynomolgus monkeys was performed by Truelove et al.(1982). However, this study had several deficiencies in its experimental design (e.g. small number of animals used in the experiment), and the results reported were either contradictory or equivocal. For example, there was only one control animal, and the three PCB-exposed animals received two different dosages. Two animals received 100 μg/kg/day for 238 days, while the third animal was administered 400 μg/kg/day for 267 days. As the authors themselves felt the study was too small and the findings too often equivocal to be relied upon (Truelove et al., 1982), this paper will not be discussed further.

Barsotti and Van Miller (1984) fed 24 female rhesus monkeys diets intended to contain 0.0, 0.25, and 1.0 ppm of Aroclor 1016. The actual dietary levels measured in the three diets were 0.005, 0.164, and 0.700 ppm of Aroclor 1016. The doses of PCBs consumed were 0.0, 4.5, and 18.1 mg/kg, respectively, over the 87 ± 9

week interval of the experiment. The mean PCB concentration in the subcutaneous adipose tissue of eight randomly selected animals averaged 0.69 ± 38 ppm in five of the animals and non-detectable in the remaining three. The levels of PCB accumulated by the animals fed PCBs are provided in Table 3.4.17. During this period, no change occurred in the general appearance of the animals, and food consumption remained at normal levels. After seven months of exposure the animals were bred. All animals carried to term and gave birth to viable offspring. The birth weights of the offspring of the high-dose group were significantly lower than those of control animals, but there were no significant differences in neonatal head circumference or in crown to rump measurements. This difference in body

Table 3.4.17

Subcutaneous Fat PCB Levels in Monkeys Fed Aroclor 1016 for 22 Months

PCBs in Diet (intended)	PCB in Subcutaneous Fat (ppm)			
	4 months	7 months	Parturition	Weaning
0.0 ppm	--	--	0.37 ± 0.16	--
0.25 ppm	1.30 ± 0.83	1.61 ±0.43	1.29 ± 0.53	1.50 ± 0.53
1.0 ppm	2.16 ± 1.1	5.03 ± 3.54	2.92 ± 0.70	4.30 ± 1.50

Adapted from Barsotti and Van Miller (1984)

weight was no longer significant by the time the animals were weaned. PCB levels measured in infant adipose tissue or the breastmilk of the lactating mothers are provided in Table 3.4.18. The data showed that the levels had appreciably decreased between four and eight months of age and that PCBs were 1.5 times higher in serum fat than in both body fat or milk fat, which were essentially equivalent. An analysis of the isomers found in these tissues, when compared to the Aroclor 1016 standard, clearly demonstrated that certain isomers were more readily eliminated than others.

Fetotoxicity in rhesus macaques resulting from the administration of 3,4,3',4'-tetrachlorobiphenyl (TCB) has been reported by McNulty (1985). In this

review article the author lists dietary levels of 100,000 ppb Aroclor 1242, 25,000 ppb of Aroclor 1248, and 1,000 ppb of either 3,4,3',4'-TCB or 3,4,5,3',4',5'-hexachlorobiphenyl as equitoxic doses in monkeys.

Table 3.4.18

Aroclor 1016 Concentrations

A. Tissues of Offspring

PCBs in Diet (intended)	PCB in Fat (ppm)		
	At birth	4 months	8 months
0.00 ppm	1.54	--	0.46
0.25 ppm	1.65	10.3	1.96
1.00 ppm	3.37	27.5	3.75

B. Breastmilk Residues (ppm)

PCBs in Diet (intended)	Month 1	Month 2	Month 3	Month 4
0.0 ppm	0.69	0.94	0.82	0.73
0.25 ppm	1.08	1.23	1.74	1.83
1.0 ppm	3.00	3.70	3.46	3.60

Adapted from Barsotti and Van Miller (1984)

Cited in this review are studies in which two monkeys were administered 3.15 mg/kg and four monkeys were administered 0.63 mg/kg in nine divided doses given between days 20 and 40 post-conception. All six monkeys had spontaneous abortions. Maternal toxicity was apparent in each monkey, but the symptoms typically did not appear until after the abortion.

3.4.5. Studies in Other Species

3.4.5.1 Guinea Pigs

In addition to the numerous studies in mice and rats already described, the reproductive effects of PCBs in other rodents, such as the rabbit and guinea pig, have also been observed. For example, Brunstrom et al. (1982b) examined the effects of prenatal PCB exposure on guinea pigs by feeding pregnant guinea pigs 2.2 mg of Clophen A50 on days 16-60 of gestation for a total dose of 100 mg per animal. This PCB exposure proved to be fetotoxic, increasing the fetal death rate from 1.5% in untreated dams to 69% in the PCB-treated animals. When the experiment was repeated using 2,2',4,4',5,5'-hexachlorobiphenyl (100 mg per animal), fetotoxicity (as indicated by a significantly higher fetal death rate) was not observed.

In a follow-up study, Kilhstrom (1982) examined the influence of fetal albumin concentration on the placental transfer of 2,2',4,4',5,5'-hexachlorobiphenyl (2,4,5-HCB). On days 60-65 of gestation, radiolabeled HCB was infused into the jugular vein of the dam while one umbilical artery and umbilical vein were connected to a recirculating perfusion system. Various perfusion media were then tested for the rate of placental transfer of 2,4,5-HCB. When heparinized guinea pig blood was the perfusion medium, the transfer of HCB from maternal blood was rapid. Radioactivity was soon found to be greater on the fetal side, and the extent of this difference was dependent upon the perfusion rate. Transportation was also rapid when bovine serum albumin/normal saline solution was used as the perfusion medium. Dose-response studies, along with tests using saline alone or an albumin-containing medium, indicated that albumin acted as the major storage site on the fetal blood side.

Hedman et al. (1985) have also examined the effects of 2,2',4,4',5,5'-hexachlorobiphenyl (2,4,5-HCB) on placental blood flow in the guinea pig. In these experiments, groups of approximately nine guinea pigs were fed

peanut oil containing doses of either 0.176 or 1.76 mg of 2,4,5,-HCB on days 45-62 of gestation. This corresponded to total doses of 3 or 30 mg of 2,4,5-HCB, respectively. This dosage regimen produced no significant effect on litter size or on fetal weight. On day 60 of gestation the guinea pigs were catheterized, and on day 63 blood flow was determined using the microspheres technique. The relative perfusion of blood was measured in the placenta, myometrium, ovaries, mammary gland, adrenals, spleen, kidneys, and lungs. Using this technique, the authors found a significant decrease in perfusion in the placenta (82% of control values) in animals of the 30 mg dose group along with a significant increase in perfusion in the lungs (311% of control values) of these animals. Given the fact that the 3 mg dose group had a relative placental perfusion that was 25% greater than the control value, the reliability and relevance of the reported decrease is difficult to ascertain from these experiments. It was noted that 8/9 placenta in the high-dose group were reported to contain small areas of discoloration and necrosis. The authors of this paper further noted that this and the reduced placental blood flow did not affect fetal weight, probably because the placenta is believed to have 20-30% more transport capacity than it needs to maintain adequate nutrition of the fetus. Yet analysis of individual animals indicated that there was a negative trend when fetal weight was correlated to HCB levels in the fat of the placenta. Because of this trend, the authors suggested that further investigation of the reported effect on placental flow was warranted.

3.4.5.2 Rabbits

Thomas and Hindsill (1980) fed female New Zealand white rabbits diets containing 0, 10, 100, or 250 ppm Aroclor 1248 for four weeks prior to mating, throughout gestation, and during lactation. The total amount of PCBs consumed by the three-kg rabbits during the entire feeding period was 0.12, 0.92, and 2.99 grams for the respective dietary levels of PCBs. There were no changes in appearance or weight gain in the treated animals. The average size of the litters, the appearance of the neonates, and the postnatal mortality were likewise not altered in the treatment groups. The offspring of the group fed 250 ppm had significantly lower birth weights and continued to remain significantly smaller through nine weeks of observation (i.e. they averaged about 1250 grams versus the

1500 gram weights of the controls). These offspring also exhibited a reduced contact-sensitivity response to dinitrofluorobenzene. There was no PCB-related immunotoxicity in any of the treatment groups, however, as measured by plaque-forming cell responses to sheep red blood cells (SRBC) or serum anti-SRBC antibody titers. Differential white blood cell counts, mitogen responsiveness of peripheral blood lymphocytes to conconavalin-A or phytohemagglutinin, and relative spleen or thymus weights were also normal in the offspring of PCB-treated dams. The lack of embryotoxicity, fetotoxicity, and toxicity in the neonates at these doses was interesting in light of the fact that the 250 ppm level is only one-half that level producing abortions and maternal mortality, and approximates the dosage level of Aroclor 1254 at which these problems begin to occur (Thomas and Hinsdill, 1980; Villeneuve et al., 1971a). For this reason, the authors concluded that the offspring are no more sensitive to PCBs than their mothers.

Consistent with the findings of this study, Villeneuve et al. (1971a&b) reported no significant effects on the total number of fetuses, number of viable fetuses, number of resorption sites, number of abortions, fetal weights, fetal liver weights, or placental weights from either Aroclors 1221 or 1254 in rabbits fed 1-10 mg/kg/day for 28 days after mating. However, when Aroclor 1254 was administered at doses of 12.5 mg/kg/day and above, abortions, stillbirths, and maternal deaths occurred. Villenueve and coworkers (1971b) also reported on the effects these two Aroclors had on microsomal enzyme activity. PCB treatment tended to lower vitamin A concentrations in maternal liver but the total amount of vitamin A present in the liver was not decreased. The levels and total amount of vitamin A in fetal liver was increased by 1.0 mg/kg Aroclor 1221 but decreased by 10.0 mg/kg Aroclor 1254. In contrast, PCB treatment had no effect on protein levels in maternal or fetal liver homogenates, but 10.0 mg/kg Aroclor 1254 did increase aniline hydroxylase and aminopyrine N-demethylase activity when expressed per gram of tissue. Given the above results the authors concluded that the NOEL for enzyme induction was between 1.0 and 10.0 mg/kg for Aroclor 1254 and above 10.0 mg/kg for Aroclor 1221.

3.4.5.3 Swine

Hansen et al. (1975) fed female pigs Aroclor 1242 at a dose level of 20 ppm throughout gestation and nursing. Untreated sows delivered 11.4 live pigs per litter while PCB-treated sows averaged only 6.4 pigs per litter, a difference significant at the 0.05% level. The treated sows' milk was found to contain less than 2 ppm PCBs on a whole milk basis. No neonatal mortality prior to weaning was reported and the weaning weight of the treated animals averaged 18.1 kg compared to the 18.8 kg of untreated control piglets. Pathologic lesions in the PCB-treated sows included shallow erosions of the gastric mucosa (2/5 of the sows), hypertrophy of the liver, and a slight atrophy of the spleen and thyroid gland. A slight atrophy of the spleen and thyroid gland was also observed in their offspring. However, confounding the results of this study, the investigators noted that chronic septicemia undoubtedly contributed to a number of the findings for the PCB-treated group.

3.4.5.4 Cows

Platanow et al. (1971) examined the transfer of PCBs to dairy products in two cows. One cow was given a single 10 mg/kg dose of Aroclor 1254 while the second received a 100 mg/kg dose. The milk from each cow was collected and pooled for four days, and the levels of PCBs found in the dairy products made from this milk are listed in Table 3.4.19.

Following the accidental ingestion of a PCB-containing waste oil (60% Aroclor 1248) by several beef cows, Peterson et al. (1983) studied the PCB residues in a lactating cow and her surrogate calf. The exposure occurred during the third trimester of the cow's pregnancy and a fat biopsy revealed levels too high to market the cow for her meat. Two months after the cow was purchased (as a heifer) for monitoring purposes, she delivered a full term calf that died of respiratory failure immediately after birth. A newborn calf born to a PCB-free cow was then introduced to the PCB-containing cow to act as a surrogate for the lost calf. The PCB tailhead fat level in the cow at the time of purchase was about 15 ppm; 60 days later this level had decreased to 11 ppm while her calf had a PCB fat level of 14 ppm

Table 3.4.19

PCB Concentrations in Dairy Products from Cows Administered a Single Dose of Aroclor 1254

Dairy Product	Cow 1 10 mg/kg PCB (ppm)	Cow 1 10 mg/kg Fat (%)	Cow 2 100 mg/kg PCB (ppm)	Cow 2 100 mg/kg Fat (%)
Whole milk	3.89	5.05%	36.10	5.65%
Cream	23.32	17.50%	262.87	22.60%
Skimmlk	0.46	0.14%	1.52	0.13%
Heated skim milk	0.17	0.14%	0.43	0.13%
Nonfat dry milk	1.67	1.65%	4.84	1.51%
Cottage cheese	1.49	1.50%	5.93	1.20%
Whey	0.28	0.03%	1.62	0.04%

Adapted from Platanow et al. (1971)

at birth. After 171 days of nursing the surrogate calf, the cow's PCB fat concentrations had dropped to 6 ppm, while the calf's fat levels had increased to almost 22 ppm. Analysis of the cow's milk demonstrated levels of 9 ppm at birth, and a constant level of about 5 ppm from day 30 to about 270 after birth. Some 600 days after giving birth, the PCB levels in the fat tissue of the cow and her surrogate calf had declined to below 2 ppm. Thus, this study demonstrated that the *in utero* transfer of PCBs leads to fat levels in the newborn that were equivalent to those of the cow. Further, as in other animals, more PCBs are transferred during lactation than during pregnancy. Lastly, the terminal elimination phase of both the cow and her surrogate calf, as it appears in Figure 1 of this paper, exhibits a half-life on the order of 150-250 days.

Perry et al. (1982;1984) have also studied the *in utero* transfer of PCBs in cows. Eighteen pregnant crossbred (Hereford x Simmental) beef cows were assigned to one of three pens. Each pen received 11.4 kg of corn silage plus 454 g of a 32% protein supplement. Each cow was thought to be approximately three months pregnant when the study was started. In one pen the cows were fed silage from a clean silo; in a second pen cows were fed silage from a silo that had been coated with a plastic containing Aroclor 1254; and in the third pen the cows were

fed clean silage to which 200 mg of Aroclor 1254 was added to the feed of each cow. After thirty days one calf was taken by Ceasarean section from one cow in each group and fat biopsies of these three cows were also taken, analyzed, and found to contain 1.20, 12.30, and 42.80 ppm PCBs, respectively. Milk samples taken from these cows, after averaging three daily samples, contained, respectively, 0.50 ppm, 9.8, and 76.7 ppm. Tissue samples taken from the calves delivered from these cows contained fat concentrations of 0.65, 18.1, and 130.6 ppm, respectively.

In the second phase of this study, the remaining five cows in each group were allowed to give birth, were taken off the silage after about 3.5 months of exposure, and were allowed to pasture with their calves. These cows were again bred, and beginning six months after the PCB exposure had been terminate these cows were slaughtered for PCB analyses. At 120 days post exposure the mean PCB fat levels in the remaining cows of groups 1, 2, and 3 were 0.80, 13.5, and 80 ppm, respectively. The average fat value for the cows slaughtered between October 15 and January 10 were 0.79, 7.80, and 38 ppm, respectively. The average fat PCB levels in the fetuses taken from these cows during this period of time was 0.34, 6.36, and 33.4 ppm.

Willett et al. (1987) have recently reported a fairly comprehensive study of the effects of PCBs on dairy cows. The effects of PCBs (Aroclor 1254) on Holsteins were monitored for a complete lactation period, a nonlactating period, and 42 days of the subsequent lactation period after having received three sequential 60 consecutive daily doses of 10, 100, and finally 1,000 mg/day of Aroclor 1254. The levels of PCBs achieved in these cows with this dosage regimen are listed below in Table 3.4.20. The concentrations of PCBs in the calves born to these cows were 0.08 ppm in blood and 26.4 ppm in fat.

During the 350 or so days of this experiment, PCB treatment did not adversely affect body weight, food intake, or milk production. In fact, compared to their previous average milk production, the PCB-treated cows produced 2.6 kg more milk while the control cows increased their milk production by only 0.9 kg. The average net energy (i.e., milk production per unit of dry matter intake) was the same in both groups during the first (1.46-vs-1.45) and second (1.36-vs-1.35)

lactation periods. The weekly clinical evaluations of heart rate, respiration rate, and body temperature did not differ between the control and PCB-treated groups. In addition, serum and urine clinical chemistry measurements revealed that no dysfunctions were caused by the PCB exposure. The gestation period of the PCB-treated cows was slightly longer than that of the control group, i.e. 284.7 ± 1.8 days versus 270 ± 1.7 days, and may have contributed to the slightly greater birth weights of the calves born to PCB-treated cows (49.2 kg versus 46.4 kg). The growth of calves from both groups was normal, and histological evaluations of the organs taken from PCB-exposed calves revealed no treatment-related abnormalities. Since the highest dose in this study generated adipose and milk fat levels of 70 and 91 ppm, and because these levels would probably require, for economic reasons, the destruction of the exposed cows, the authors concluded that there was little need for additional studies to define the amount of PCBs producing toxicity in cattle.

Table 3.4.20

PCB Levels in Cows Given Aroclor 1254

Experimental period	PCB Concentrations (ppm)		
	Plasma	Milk fat	fat
End of 10 mg/day	0.005	1.9	1.4
End of 100 mg/day	0.021	10.9	6.9
End of 1000 mg/day	0.140	91.3	70.0
End of experiment	0.045	3.1	17.7

Adapted form Willett et al. (1987)

3.4.5.5 Birds

In addition to the studies in mammals, the reproductive toxicity of PCBs has also been studied in birds. Tumasonis et al. (1973) reported the effects of feeding Aroclor 1254 to White Leghorn chickens at a dietary level of 50 ppm for six weeks.

This level of exposure of PCBs did not affect the fertility of the hens during the six weeks of exposure or during the following 20-week observation period. However, egg production by PCB-exposed hens was temporarily decreased during weeks 3-12 of the observation period. The hatching percentage had dropped significantly by week two of the PCB exposure interval. It remained at 0% from week three of exposure through week nine of the observation period and did not return to normal levels until week 17 of the observation period. Incubation of the eggs indicated that gametogenesis and fertilization were not affected, but the levels of PCBs that had accumulated in the eggs proved to be embryotoxic. As the level of PCBs in the eggs increased, embryonic development was arrested at progressively earlier stages of development. When PCB levels in the egg yolk reached 10-15 ppm or greater, physical deformities were also evident. Measurement of the PCB concentration in yolks revealed that by week two the levels were about 72 ppm; by week six of the exposure the level of PCBs present in eggs had reached 200 ppm. During the observation period the accumulation of PCBs in eggs steadily declined and by week 17 PCB levels in the yolk had declined sufficiently such that adverse effects on embryonic viability were no longer observed. In addition to the reported levels of PCB accumulation in eggs, the PCB levels in two chickens that died during weeks 7 and 13 of the observation period were also provided. The PCB levels measured in these two chickens were 56.2 and 42.5 ppm for liver, 52.7 and 20.0 ppm for brain, and 12.0 and 3.2 for muscle. It was concluded that the evidence from this experiment suggested that PCB excretion via the egg is substantial and may result in a significant decline in bird populations if PCB levels in the egg reach those associated with embryotoxicity.

Cecil et al. (1974) performed a similar study in the White Leghorn chicken by examining the adverse effects produced by dietary PCBs levels of the following concentrations: 2 ppm of Aroclors 1242, 1248, and 1254, or 20 ppm of Aroclors 1221, 1232, 1242, 1248, 1254, 1268, and 5442. Chickens were fed diets containing these levels of PCBs for nine weeks, followed by a seven-week observation period. Aroclors 1232, 1242, 1248, and 1254 at 20 ppm caused a decline in hatchability. The 2 ppm exposure to Aroclors 1242, 1248, and 1254, or higher (20 ppm) exposures to Aroclors 1221, 1268, or 5442 had no effect on hatchability. Thus, the adverse effects reported in this study could not be correlated to the chlorine content of the mixture

being tested. While the data presented in this report are virtually devoid of statistical analysis, exposure to some of the commercial PCB mixtures appeared to result in an increase in embryo mortality and in fetal abnormalities such as edematous cysts of the rump and subcutaneous edema about the neck and rump.

Ahmed et al. (1978) have tested the male reproductive toxicity of Aroclor 1254 in the White Leghorn. Even though roosters fed diets containing PCB levels of 0, 10, 20, and 40 ppm for a period of 40 weeks had lower semen volume, semen concentrations, and testes weights, there were no adverse effects on fertility, hatching percentage, body weights, feed consumption, or mortality.

Haseltine and Prouty (1980) fed mallard ducks a diet containing 150 ppm Aroclor 1242 for an average of 33 days prior to the time egg laying started and for a total of 12 weeks during the laying season. No adverse effects on the time to lay a clutch of eggs, fertility, embryo mortality, or hatching success were noted. Eggshell thickness was decreased 8.9% by the PCB diet, a change which represented a significant difference. The third egg laid by each hen was collected for PCB analyses and the results of this experiment revealed the average PCB concentration of these particular eggs (measured as Aroclor 1260) was 105 ppm PCBs. Despite this concentration of PCB in the egg, no difference in survival or weight gain in the ducklings was observed. Thus, the results of this study suggest that mallards are considerably more resistant to PCBs than is the White Leghorn chicken.

Similarly, McLane and Hughes (1980) fed screech owls (*Otus asio*) a diet which contained 3 ppm of Aroclor 1248. In the first study the owls were given this diet for eight weeks prior to the onset of egg laying. At the time of hatching the diet was changed to whole rodents to allow the chicks to be raised to fledglings. In the second study, the adult owls from the first study were again placed on this diet 60 days after the first set of fledglings were hatched and maintained on this diet through the next laying season. No significant changes were observed in the number of eggs laid, in eggshell thickness, in the number of young hatched, or in the number of young fledged. The PCB residues measured in eggs that were broken or became addled while the adult birds attempted to hatch them ranged

from 2.24 to 4.84 ppm in the first experiment and from 3.9 to 17.8 ppm in the second experiment. The PCB residues in the carcasses of 10 fledglings sacrificed during these experiments were only 0.29-0.98 ppm in the first experiment and 0.96-2.16 ppm in the second experiment. In contrast, the carcasses of two adult birds sacrificed at the end of these experiments contained PCB residues of 10.6 and 15.0 ppm. Thus, it does not appear that the amount of PCBs transferred to the eggs of screech owls is as great as that transferred by chickens.

The reproductive effects of PCB on another raptor, the American kestrel (*Falco sparverius*), were studied by Bird et al. (1983). Kestrels were fed cockerels that had been injected with Aroclor 1254 to produce an effective daily dietary level of 33 ppm. Aroclor 1254 produced a decrease in sperm concentration without compensatory increase in semen volume. As a consequence there was a 22-27% decline in sperm numbers per ejaculate while sperm motility and testicular mass were unaffected. The authors concluded that the effects of these changes on free-living kestrels would be unknown, however, given the negative results of Ahmed et al. (1978). The corresponding PCB residues in various tissues of the control group were non-detectable for liver, 0.41 ppm for muscle, and 1.03 ppm for the testes. In the birds fed diets containing PCBs, these tissues contained residue levels of 91.6 ppm, 107.3 ppm, and 127.9 ppm, respectively.

Tori and Peterle (1983) have examined the effects of Aroclor 1254 on the courtship behavior of the morning dove. Doves that had previously laid at least one fertile clutch were fed diets containing either 0, 10, or 40 ppm of Aroclor 1254 for 42 days. Two weeks later the doves were paired and observed for the next 30 days. PCB treatment increased the length of the courtship phase, in a dose-related manner, from an average of 11.3 days in control birds to 22.1 and 30 days. There were no significant differences between control birds and birds fed 10 ppm in the length of time or behavior scores for the pair-bond formation period. In fact, birds fed PCBs paired approximately four days sooner than did control birds. In contrast to a more rapid pair-bonding, birds fed the 10 ppm diet spent some 8.5 days longer in the courting period and only 4/8 birds nested while 8/8 control birds nested. However, when only the pairs that nested were compared, PCB-treated birds still spent seven days longer at nest initiation, a factor which delayed egg laying as

well.

In a similar study, Koval et al. (1987) examined the effect of PCB feeding on mourning doves live-trapped in Ohio. Dove pairs that laid eggs in the observation period before the experiment were fed 10 ppm Aroclor 1254 during an isolation period of 28 days. Nesting behavior was observed and plasma progesterone levels were determined at various time intervals. Only 50% of the PCB-treated birds laid eggs compared to 77% of control birds. PCB treatment also appeared to significantly delay on-nest behavior and oviposition, and extended the time on-nest. Serum progesterone levels in PCB-treated females were consistently slightly lower than controls, and peak progesterone levels before oviposition appeared to shift one to two days earlier with PCB treatment while a second peak occurring after oviposition was not seen in PCB-treated birds. The authors concluded that this evidence suggested a delayed reproduction in PCB-treated mourning doves.

Although Koval et al. (1987) concluded that PCB treatment delayed reproduction, several aspects of their study suggest that such a conclusion is not warranted. First, the birds used in the study were live-trapped in Ohio, in the Great Lakes region which is known to have considerable environmental PCB contamination. Thus, the authors had no indication of previous PCB exposure of their experimental animals, nor did they attempt to measure serum PCB and pesticide levels to monitor for the confounding influences of previous toxic exposures. Second, although the authors noted a 27% decrease in eggs laid and a slight delay in nesting behavior in PCB-treated birds, they did not indicate what effect the prolonged captivity of doves had on their reproductive success and nesting behavior. It is quite possible that such factors are highly variable and dependent upon a number of other factors (season, temperatures, nutrition, feed palatability, previous exposures, etc.) which are difficult to accurately assess in such small groups of birds as were used in their study (10 birds/treatment). Third, the progesterone levels in PCB-treated birds were slightly but not significantly lower than controls, and the apparent shift of peak progesterone levels by one to two days earlier in PCB-treated birds suggests a more rapid reproduction, in contrast to the authors' suggested delay. And finally, the pertinence of the finding

that control doves showed a second progesterone peak at three to four days after oviposition while PCB-treated doves did not is unknown and was not discussed by the authors. Therefore, although this study noted changes in nesting behavior, oviposition, and progesterone cycles in PCB-treated mourning doves compared to control birds of unknown exposure, no particular finding can be attributed to PCB exposure in view of the uncontrolled variables and/or unknown aspects influencing their findings.

McArthur et al. (1983) also examined the effects of PCBs on the breeding success of doves, but the results of this study are limited by the fact that the birds were fed a diet which also contained DDE, mirex, and photomirex. Ring Doves were fed either a control diet, a diet containing 8 ppm Aroclor 1254, 1.67 ppm DDE, 0.297 ppm mirex, and 0.0954 ppm of photomirex (referred to as the low-dose diet), or a diet containing 28.03 ppm Aroclor 1254, 4.61 ppm DDE, 0.897 ppm mirex, and 0.324 ppm photomirex (high-dose diet). The birds were fed these diets for a 90-day isolation period, to allow for gonadal regression, then separated and paired with a member of the opposite sex and fed the diet they received during isolation during one complete reproductive cycle. After the birds had fledged their squabs (considered fledged at 14 days of age), the adults were sacrificed, and the mesenteric fat pad was removed for residue analysis. The low-dose diet resulted in fat residue levels of 120 ppm of DDE, 739 ppm PCBs, 40 ppm of mirex, and 12.7 ppm of photomirex in male birds. The high-dose diet resulted in residues of 220 ppm DDE, 1545 ppm PCBs, 58 ppm mirex, and 19.4 ppm of photomirex in the male birds. The levels in female birds were the same except for a slightly lower accumulation of PCBs and mirex in the low-dose females. The effects of this organochlorine mixture on the reproductive cycle and certain behavioral patterns are provided in Table 3.4.21. In addition to the changes listed in this table, these investigators also observed decreases in androgen levels in male birds and in estrogen levels in female birds four days post-pairing. At the mid-incubation interval the average thyroxine level of the organochlorine-fed birds was significantly increased. These changes may be of considerable interest concerning those areas of the environment in which the combined organochlorine contamination is high. Since it cannot be determined from this study whether or not these results can be extrapolated to situations which involve only PCBs, and

Table 3.4.21

Variations in the Reproductive Cycle, Parental Behavior and Reproductive Success of Ring Doves Fed A Mixture of Organochlorine Compounds

Parameter Measured	Control	Low-dose	High-dose
A. Phase of Reproductive Cycle			
Courtship[a]	0-13	0-19*	0-31*
Median clutch completion[a]	10	12.5*	19*
Incubation[a]	9-25	9-27*	13-41*
Brooding[a]	24-29	23-42	27-48
Overlap	12.8%	33.3%	58.3%
B. Parental Behavior			
Incubation attentiveness	99.4%	99.5%	96.8%
Percent time spent brooding	89.7%	89.6%	82.1%*
Percentage of absences	40.0%	43.0%	53.0%*
Percent time spent feeding young	3.2%	3.8%	1.9%*
C. Reproductive Success			
Pairs laying eggs	10/10	8/9	9/10
Eggs laid per pair	2.00	1.78	1.70
Hatching percentage	43%	49%	45%
Young fledged per young hatched	17/17	13/14	7/13*

[a] Days post-pairing
Adapted from McArthur et al. (1983)
* $p < 0.05$

given the fact that the number of young fledged per nesting attempt was only decreased by the high-dose organochlorine diet, it would appear that the changes reported in this study are of limited value for assessing the reproductive hazards of PCB exposure to wild birds.

Hoffman et al. (1986) measured the PCB and DDE residues in the eggs of black-crowned herons taken from either the San Francisco Bay National Wildlife Refuge (SFBNWR) or the Patuxent Wildlife Research Center. The eggs taken from herons living in the SFBNWR had a geometric mean PCB residue level of 4.1 ppm with values ranging from as low as 0.8 ppm to as high as 52.0 ppm. This corresponded to a slightly shorter crown-rump length and femur length as well as a slightly lower embryo weight in the contaminated embryos. Measurements that were not significantly different between the two groups include the following: egg weight, embryo + yolk sac weight, yolk sac weight, brain weight, liver weight, humerus length, radius-ulna length, and tibiotarsus length. The decreased embryo weight reported in this study appears to be related to the greater yolk sac weight of the contaminated eggs. Not only does the increase in this parameter account for much of the difference in embryo weight, but there was also no difference in egg weight or embryo + yolk sac weight between the two groups. Thus, even though the authors reported that embryonic weight was negatively correlated with egg PCB content, given the weak correlation coefficient (r^2= 0.3667) for this relationship, this interpretation of their data remains open to question. The authors further concluded that the decreased crown-rump length (a 4% change) and the decreased femur length (a 7% change) represented impaired embryonic growth. Again, however, the lack of significant changes in other bone length measurements or in organ weights argues against an impairment of embryonic growth.

Brunstrom (Brunstrom and Darnerud, 1983) had previously found that either a 20 or 100 µg/kg dose of 3,3',4,4'-tetrachlorobiphenyl (TCB) produced complete embryolethality when injected into the yolk of eggs incubated for four days, while a 4 mg/kg dose of this PCB congener only minimally affected the hatching rate. In a similar set of experiments, Brunstrom and Reutergardh (1986) injected a 100 µg/kg dose of TCB into mallard eggs and 1,000 µg/kg dose of TCB into the eggs of